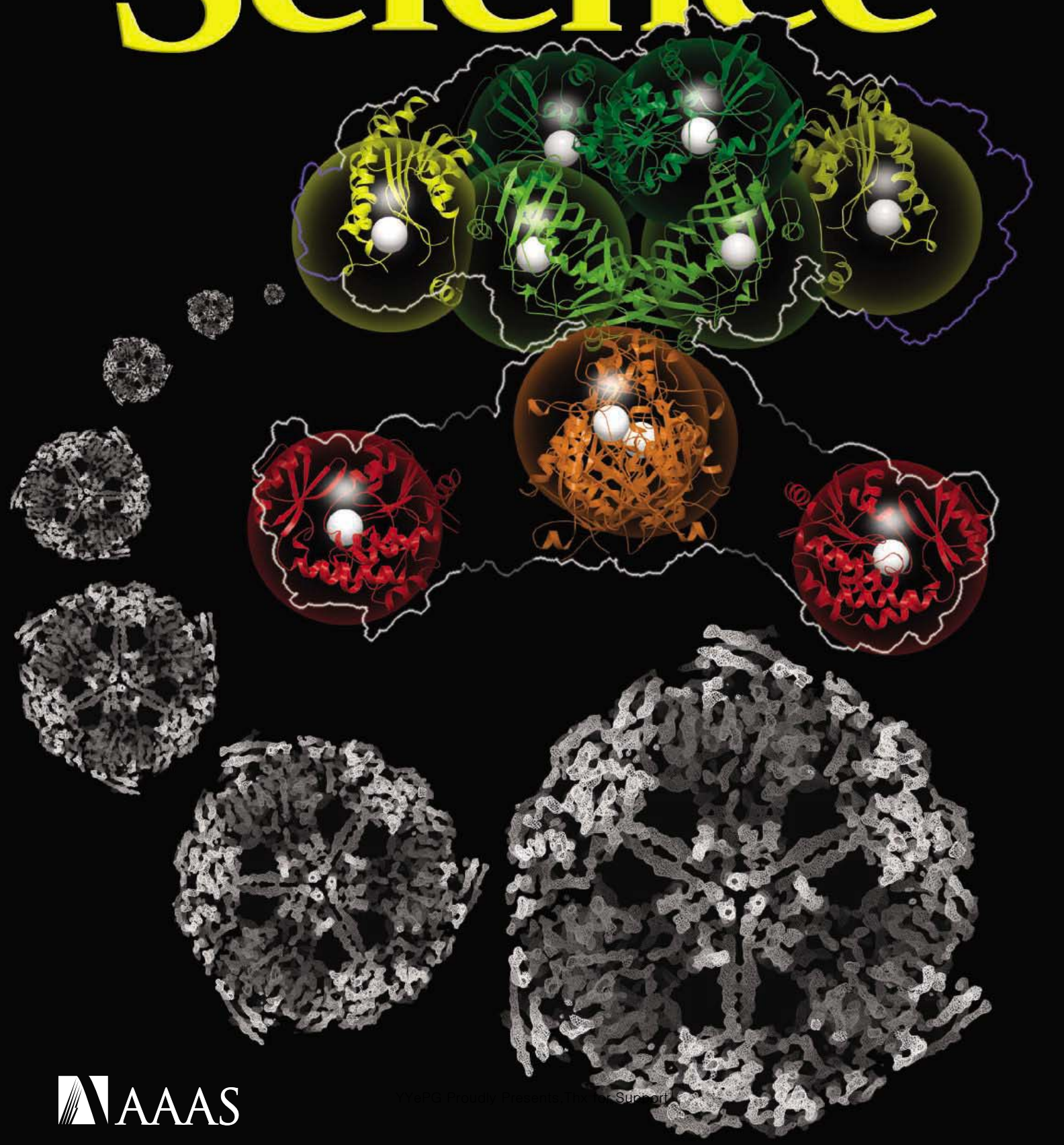
3 March 2006 | $10

Science



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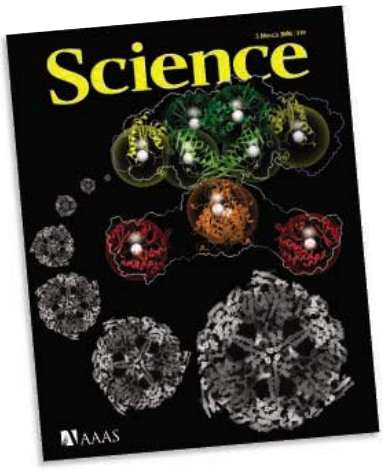


COVER

The enzymes that catalyze fatty acid formation are large protein complexes with multiple active sites. The architecture of the mammalian multienzyme (catalytic domains in various colors) is quite different from that of the fungal fatty acid synthase (in gray). Nonetheless, they catalyze the same conserved reaction pathway. See pages 1258 and 1263.

Image: S. Jenni and T. Maier

DEPARTMENTS

1207 *Science* Online
1209 This Week in *Science*
1214 Editors' Choice
1218 Contact *Science*
1221 NetWatch
1223 Random Samples
1239 Newsmakers
1304 New Products
1305 *Science* Careers

EDITORIAL

1213 The Mailbag
by Donald Kennedy

NEWS OF THE WEEK

As H5N1 Keeps Spreading, a Call to Release More Data 1224
Evidence Points to Migratory Birds in H5N1 Spread 1225
DOE Hits Potholes on the Road to Systems Biology 1226
Canadian Editors Fired in Row With Association 1226
Despite a Chilly Reception, the 'European MIT' Advances 1227
SCIENCESCOPE 1227
NSF Presents the Wide World of Science 1228
Indian Chemist Receives a Visa and an Apology 1229
Protesters March to a Different Drummer 1229

NEWS FOCUS

The Lost World of the Kihansi Toad 1230
Great Balls of Fat 1232
Getting Women Scientists Back on the Career Track in Japan 1235
A $214 Billion Plan of Action
A Passion for Teaching Leads to Engineering Change in Schools 1237

LETTERS

Crucial Choices for the Nascent ERC *Initiative for Science in Europe* 1240
Objectivity in Science *K. R. Gordon*
Influenza Mutation from Equine to Canine *M. von Grotthuss and L. Rychlewski*
Response *P. C. Crawford* et al.
CORRECTIONS AND CLARIFICATIONS 1242

BOOKS *ET AL.*

René Dubos, Friend of the Good Earth 1243
Microbiologist, Medical Scientist, Environmentalist
C. L. Moberg, reviewed by J. Strick
Civilized Life in the Universe 1244
Scientists on Intelligent Extraterrestrials
G. Basalla, reviewed by M. Shermer

POLICY FORUM

A National Tuberculosis Archive 1245
D. Gessler et al.
A Portfolio Model of Drug Development for Tuberculosis 1246
S. W. Glickman et al.

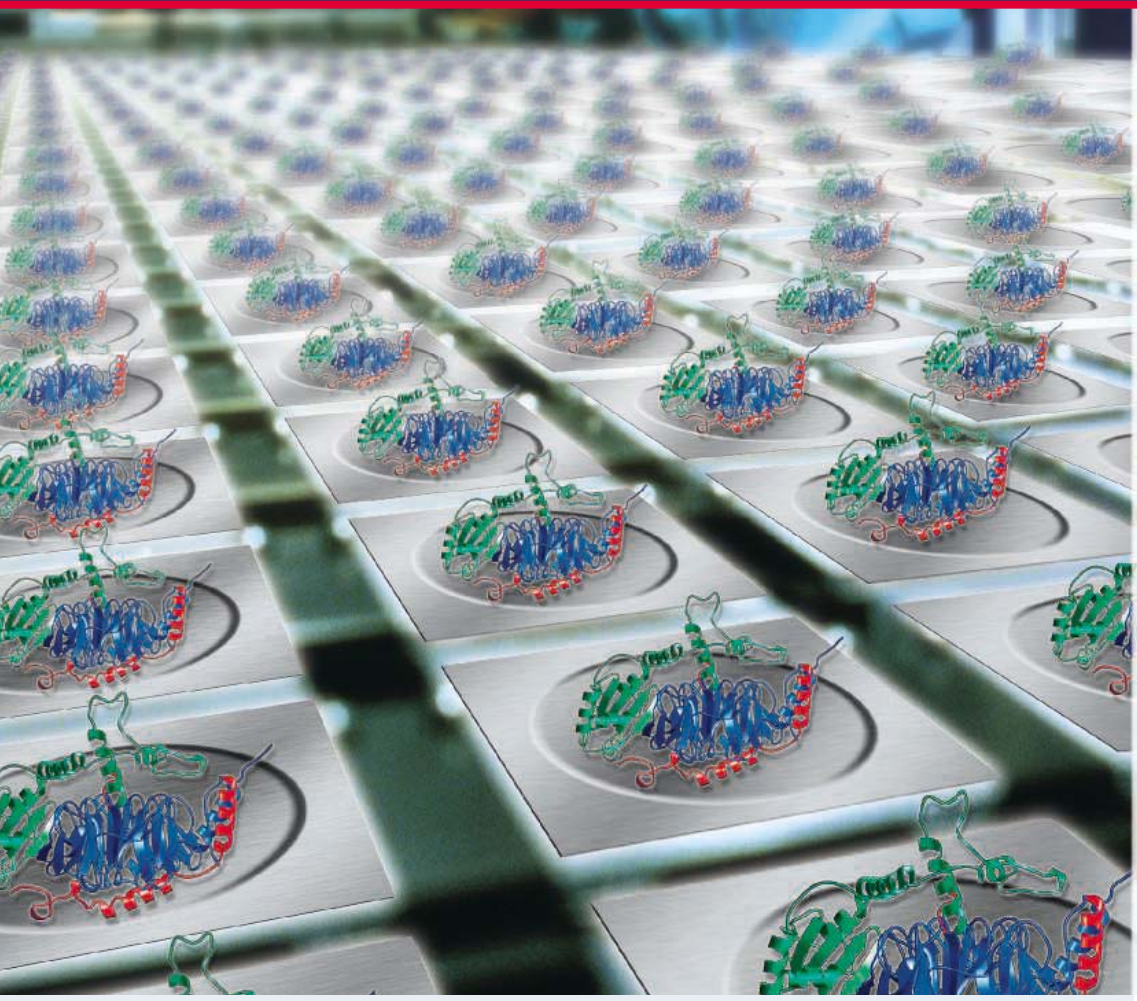
PERSPECTIVES

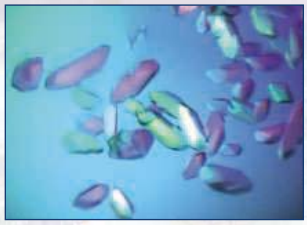
Who Are More Helpful, Humans or Chimpanzees? 1248
J. B. Silk
>> Reports pp. 1297 and 1301
An Example of Preclassic Mayan Writing? 1249
S. D. Houston
>> Report p. 1281
Creep and Flow on the Icy Moons of the Outer Planets 1250
P. R. Sammonds
>> Report p. 1267
Architectural Options for a Fatty Acid Synthase 1251
S. Smith
>> Research Articles pp. 1258 and 1263

CONTENTS continued >>

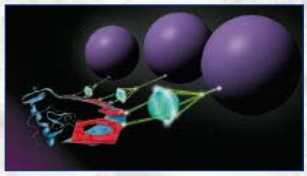
Systems Biology — Proteins and Proteomics

Standardized solutions for proteins





E. coli gyrase A C-terminal domain crystals. Courtesy of Alex Ruthenburg from Prof. Verdine's laboratory, Harvard University, Boston, USA.



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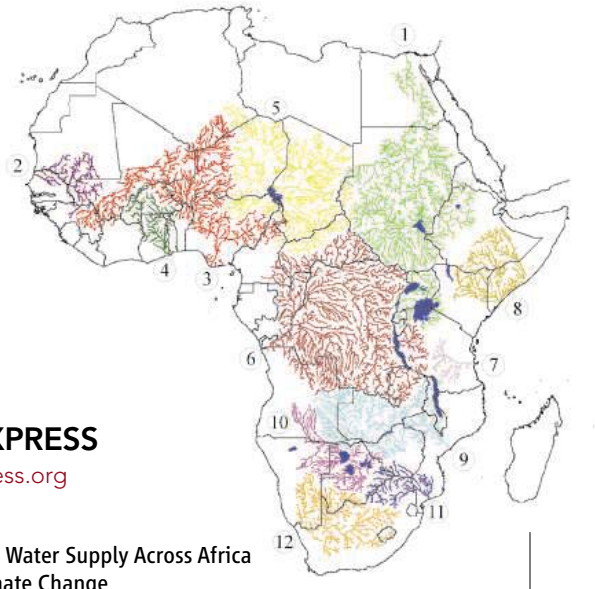

SCIENCE EXPRESS

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CLIMATE CHANGE

Changes in Surface Water Supply Across Africa with Predicted Climate Change

M. de Wit and J. Stankiewicz

Simulations of future precipitation imply that reduced stream flow will further restrict water availability across much of sub-Saharan Africa over the next century.

10.1126/science.1119929

CLIMATE CHANGE

Measurements of Time-Variable Gravity Show Mass Loss in Antarctica

I. Velicogna and J. Wahr

Satellite measurements of Earth's gravity reveal that the mass of ice in Antarctica decreased from 2002 to 2005, mainly from losses in the West Antarctic Ice Sheet.

10.1126/science.1123785

CHEMISTRY

Probing Proton Dynamics in Molecules on an Attosecond Time Scale

S. Baker et al.

Nuclear motion in H_2 and methane could be clocked less than a femtosecond after ionization by analysis of the photons released through electron-ion recombination.

10.1126/science.1123904

IMMUNOLOGY

Evidence for a Functional Second Thymus in Mice

G. Terszowski et al.

Mice have a second thymus in the neck that contributes functional T cells to the immune system, forcing a rethinking of previous experiments that assumed a single thymus.

10.1126/science.1123497

IMMUNOLOGY

Naïve and Memory CD4$^+$ T Cell Survival Controlled by Clonal Abundance

J. Hataye, J. J. Moon, A. Khoruts, C. Reilly, M. K. Jenkins

Clonal subpopulations of immune T cells—each of which binds to a different antigen—are more stable if they contain smaller numbers of cells.

10.1126/science.1124228

REVIEW

NEUROSCIENCE

Auxiliary Subunits Assist AMPA-Type Glutamate Receptors 1253

R. A. Nicoll, S. Tomita, D. S. Bredt

BREVIA

MEDICINE

Cellular Senescence in Aging Primates 1257

U. Herbig, M. Ferreira, L. Condel, D. Carey, J. M. Sedivy

As baboons age, cells that have become irreversibly senescent accumulate in various tissues, likely contributing to the aging of the whole animal.

RESEARCH ARTICLES

STRUCTURAL BIOLOGY

Architecture of Mammalian Fatty Acid Synthase at 4.5 Å Resolution 1258

T. Maier, S. Jenni, N. Ban

Architecture of a Fungal Fatty Acid Synthase at 5 Å Resolution 1263

S. Jenni, M. Leibundgut, T. Maier, N. Ban

The large multiprotein complexes that synthesize fatty acids in mammals and fungi have radically different architectures.

>> Perspective p. 1251

REPORTS

PLANETARY SCIENCE

Grain Size–Sensitive Creep in Ice II 1267

T. Kubo, W. B. Durham, L. A. Stern, S. H. Kirby

Experiments show that grain size influences the deformation speed of ice under high pressure, modifying models of the evolution and internal dynamics of icy moons.

>> Perspective p. 1250

APPLIED PHYSICS

Giant Electrocaloric Effect in Thin Film $PbZr_{0.95}Ti_{0.05}O_3$ 1270

A. S. Mischenko et al.

Application of an electric field to a thin lead zirconium titanium oxide produces a surprisingly large drop in its temperature, suggesting a potential refrigeration technology.

MATERIALS SCIENCE

Surface Self-Organization Caused by Dislocation Networks 1272

K. Thürmer, R. Q. Hwang, N. C. Bartelt

The organization of silver ions on a ruthenium surface depends on dislocations below the surface and not on strain or surface tension as had been thought.

CONTENTS continued >>

REPORTS *CONTINUED...*

MATERIALS SCIENCE

Synthesis and Characterization of the Nitrides of Platinum and Iridium 1275
J. C. Crowhurst et al.
A platinum nitride produced at high pressure has a simple structure, and an iridium nitride can persist at ambient conditions and may be nearly as stiff as diamond.

CHEMISTRY

The Rotational Spectrum of the Water–Hydroperoxy Radical (H_2O–HO_2) Complex 1278
K. Suma, Y. Sumiyoshi, Y. Endo
Microwave spectroscopy reveals that a H_2O–HO_2 complex is pentagonal, offering a signature with which to probe its postulated role in atmospheric chemistry.

ANTHROPOLOGY

Early Maya Writing at San Bartolo, Guatemala 1281
W. A. Saturno, D. Stuart, B. Beltrán
Hieroglyphic writing adorns a buried stone building in the Maya temple of San Bartolo, Guatemala, dated to about 250 B.C., closer to when writing emerged in the New World.
>> Perspective p. 1249

EVOLUTION

Toward Automatic Reconstruction of a Highly Resolved Tree of Life 1283
F. D. Ciccarelli et al.
Sequences of 36 genes in each of 191 diverse species allow construction of a highly resolved phylogenetic tree, which, when lateral gene transfer is eliminated, clarifies the tree of life.

GENETICS

Germline Mutations in Genes Within the MAPK Pathway Cause Cardio-facio-cutaneous Syndrome 1287
P. Rodriguez-Viciana et al.
Mutations that functionally alter an intensely studied cellular signaling pathway are found in young patients with a developmental delay disorder.

NEUROSCIENCE

Combined Analog and Action Potential Coding in Hippocampal Mossy Fibers 1290
H. Alle and J. R. P. Geiger
Synapses at one end of a neuron can be affected by graded synaptic currents at the other end, 0.5 millimeters away, suggesting that analog information is unexpectedly used in the brain.

BIOCHEMISTRY

Chemical Rescue of a Mutant Enzyme in Living Cells 1293
Y. Qiao, H. Molina, A. Pandey, J. Zhang, P. A. Cole
Abnormal cells harboring a mutant signaling enzyme found in some cancers can be rapidly rescued by the small molecule imidazole, suggesting a therapeutic application.

PSYCHOLOGY

Chimpanzees Recruit the Best Collaborators 1297
A. P. Melis, B. Hare, M. Tomasello
Like humans, chimps will preferentially recruit especially skilled species-mates to solve difficult problems.
>> Perspective p. 1248

PSYCHOLOGY

Altruistic Helping in Human Infants and Young Chimpanzees 1301
F. Warneken and M. Tomasello
Toddlers can recognize that an adult needs help with a task and assist, indicating empathy and altruism; young chimpanzees do the same, but less effectively.
>> Perspective p. 1248

Working the Systems 1305
A Meeting of Minds, Expertise, and Imagination 1306

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CONTENTS continued **>>**

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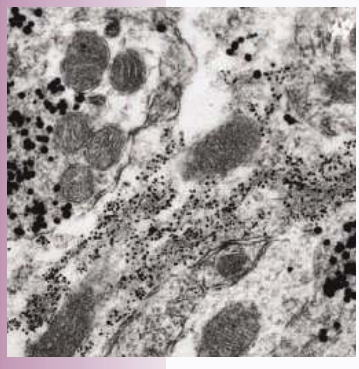

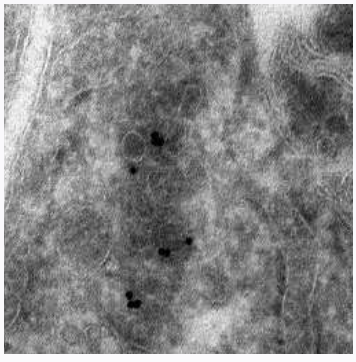

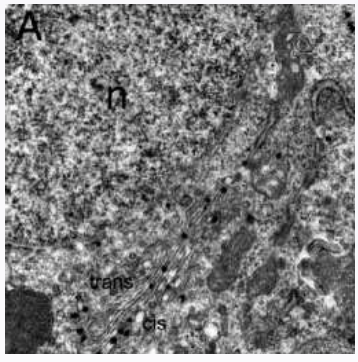

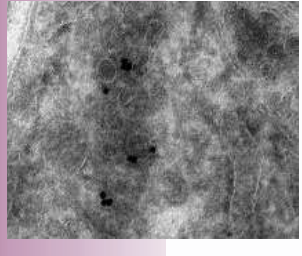

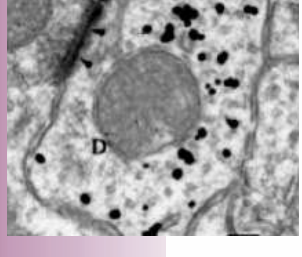

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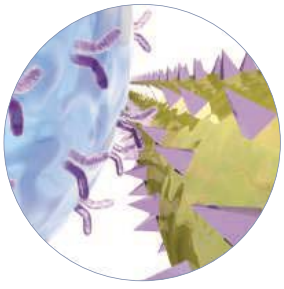

Axon and Schwann cell interact.

SCIENCE'S **STKE**

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PERSPECTIVE: Neuregulin-1 and Myelination
G. Lemke
Axonal neuregulin-1 appears to drive every aspect of Schwann cell differentiation.

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E. M. Landau
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Heat shock extends life span of yeast, worms, and flies.

MEETINGS AND EVENTS
American Aging Association meeting in June will focus on interventions in aging and age-related diseases.

SPECIAL ONLINE CONTENT

SCIENCE **CAREERS**

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

GLOBAL: Careers in Systems Biology—Special Issue Index
A. Forde
Systems biology requires a sophisticated suite of mathematical, computational, and experimental tools.

CANADA: Ottawa's Institute of Systems Biology
A. Fazekas
This institute applies systems biology tools to the study of human diseases.

SPAIN: From Molecular to Systems Biology
E. Pain
New integrative tools and genomics data have allowed Ildefonso Cases to follow his career ambitions.

GLOBAL: Systems Biology Initiatives
A. Forde
Here's a listing of major systems biology projects, training opportunities, and conferences, in the United States and Europe.

>> Careers Features pp. 1305 and 1306

SCIENCE **CAREERS**

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US: The State of the Union
B. Benderly
Two years after the ratification of the first postdoc union contract, things continue to go well at the University of Connecticut Health Center.

MISCINET: Earth Watcher, Earth Teacher
A. Sasso
Ken Ridgway, a professor at Purdue University and Lenape Tribe member, studies Earth's fundamental riddles and makes minority students feel welcome.

GRANTSNET: March 2006 Funding News
J. Fernandez
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Colonies Visible after 8 hours	✓	–	–	–	–
Endonuclease I Deficient	✓	✓	✓	✓	✓
Protease Deficient	–	–	✓	✓	–
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M13 Phage Capable (F^+)	✓	✓	–	✓	–
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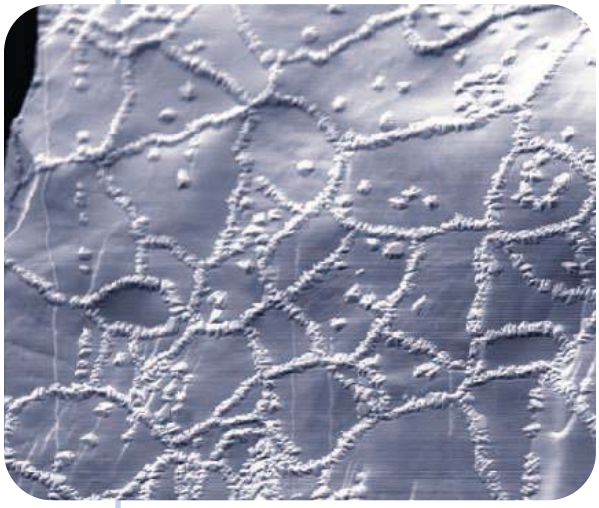


Freeze and Squeeze

Ice is a major component of the upper mantles of medium- to large-sized moons of the outer solar system, and in order to model heat flows in these bodies, it is necessary to understand how ice phases that form at higher pressures respond to stress. **Kubo *et al.*** (p. 1267; see the Perspective by **Sammonds**) perform cold-temperature experiments to address the microstructure deformation mechanism that dominates microcrystalline ice II, which was formed by overpressurizing normal ice to 300 megapascals at temperatures below 220 K. At low strain rates, the authors find that the creep mechanism becomes sensitive to grain size; smaller grains (6 versus 40 micrometers) created a weaker ice.

Synaptic Stargazin

The family of AMPA subtype glutamate receptors plays an important part in normal excitatory synaptic transmission and is also heavily involved in plastic synaptic changes. Recently, a family of homologous small transmembrane AMPA receptor regulatory proteins (TARPs), exemplified by the protein stargazin, have been discovered that regulate AMPA receptor trafficking and determine native AMPA receptor gating. **Nicoll *et al.*** (p. 1253) review how TARPs control AMPA receptors during normal synaptic transmission and during the induction of synaptic plasticity.

Two Ways to Make the Fat

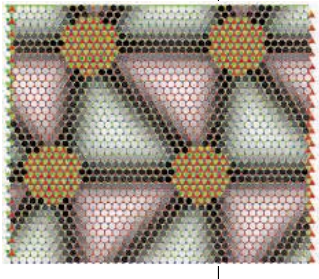
The biosynthesis of fatty acids is a central metabolic pathway in which long hydrocarbon chains are built by adding two-carbon units in a repetitive sequence of reactions (see the cover and the Perspective by **Smith**). **Maier *et al.*** (p. 1258) and **Jenni *et al.*** (p. 1263) present the detailed views of the mammalian and fungal fatty acid synthase complexes by fitting the homologous catalytic domains from the corresponding bacterial enzymes into 4.5 or 5.0 angstrom electron density maps. Amazingly, the seven functional domains are arranged in completely different ways. The mammalian complex resembles an "X" in which the arms flex upward and downward during each round of addition. The fungal enzyme looks like an "egg" with separate reaction chambers in the top and bottom halves.

Giant Electrocaloric Effect

One route to improved energy efficiency is to put waste heat to use, and electrocaloric materials could in principle use waste heat to power refrigeration. The application of a electric field across an electrocaloric material has been known for several decades to reduce its temperature, but the effect was too small in these materials to allow commercial applications. **Mischenko *et al.*** (p. 1270) now show that a perovskite thin-film material exhibits an electrocaloric effect about two orders of magnitude larger than previously found in other materials.

Order Out of Misfits

The origins of the stability of a self-ordered array of defects have been determined by an analysis of the thermal fluctuations of their positions. **Thürmer *et al.*** (p. 1272) reexamined hexagonal arrays of sulfur-induced vacancy islands in a partial silver monolayer on the Ru(0001) surface by taking time-series scanning tunneling microscopy images at different temperatures. An analysis of how neighboring islands fluctuate parallel and perpendicular to the line connecting two islands allowed the stiffness and restoring forces operating on island-island bonds to be determined. The stability of this array is determined by the arrangement of misfit dislocations within the film, which themselves arose during the self-assembly processes.



Hard Noble Nitrides

Recently, a platinum nitride was synthesized under high pressure and temperature and shown to possess a large bulk modulus, but the structure of the compound was unknown. **Crowhurst *et al.*** (p. 1275) report that this material has a stoichiometry of PtN_2 and that the structure is similar to that of pyrite. Under similar conditions, they could synthesize a recoverable nitride of iridium. Despite the similar stoichiometry of this compound, it has a much lower structural symmetry.

Early Writing on the Walls

Writing has been thought to have emerged in the New World in the Olmec culture, or more broadly near Oaxaca; clear evidence is seen in these regions by about 300 B.C., and some finds suggest an origin one to three centuries earlier. Aside from a few hints, clear writing in Maya ruins was enigmatically found only for much later dates. **Saturno *et al.*** (p. 1281, published online 5 January; see the Perspective by **Houston**) now describe a series of hieroglyphs from a deep room in a Maya temple that was built between 200 and 300 B.C. Writing appeared to emerge in the Maya region near the time when it appeared widely elsewhere in Mesoamerica.

Finding Branches of the Tree of Life

In order to understand how evolution occurred, from the development of molecular networks to organ systems and the relationships of organisms, it is necessary to have a framework.

Continued on page 1211

CREDITS (TOP TO BOTTOM): KUBO *ET AL.*; THÜRMER *ET AL.*

SCIENCE & ENGINEERING

VISUALIZATION CHALLENGE

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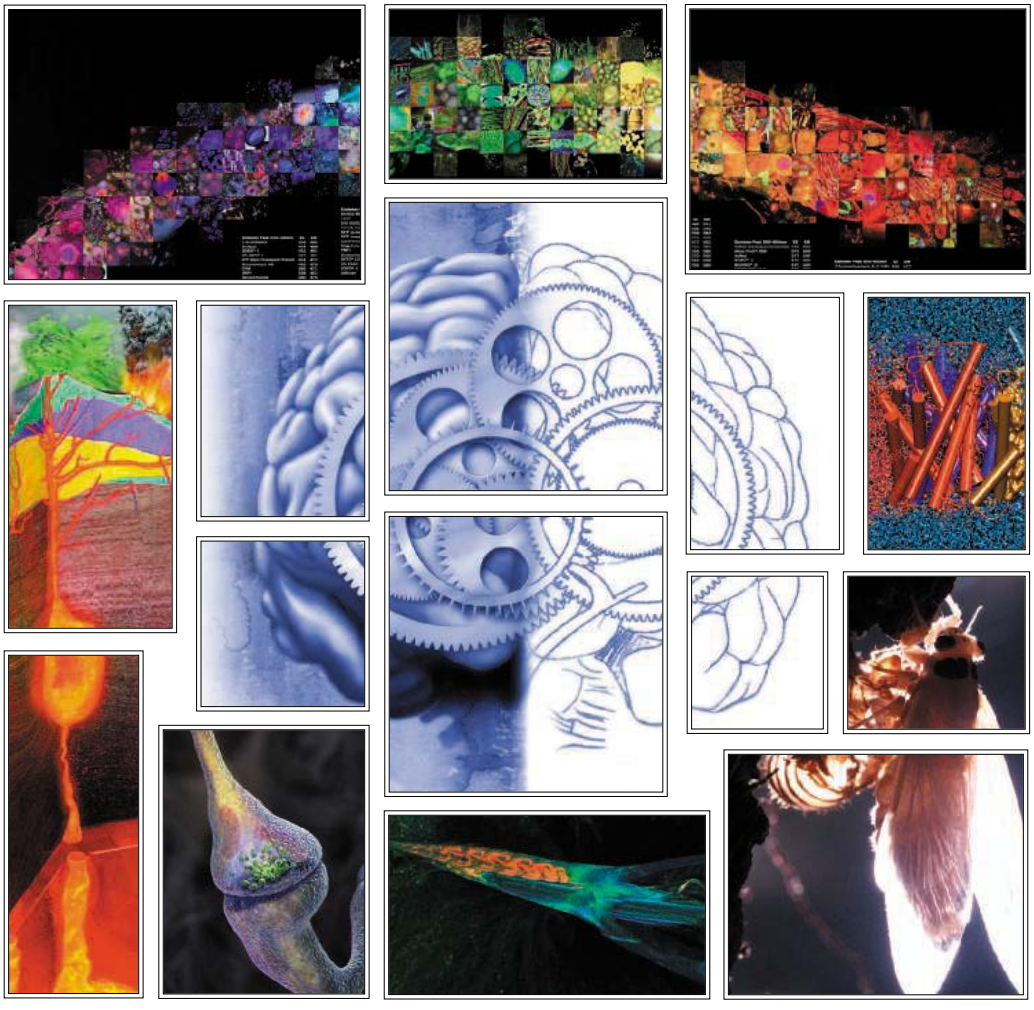

SCIENCE AND ENGINEERING'S MOST POWERFUL STATEMENTS ARE NOT MADE FROM WORDS ALONE

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ENTRY DEADLINE: MAY 31, 2006

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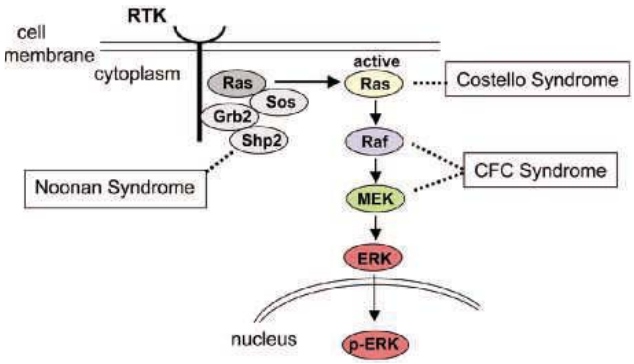
Complete Entry Information: www.nsf.gov/news/special-reports/scivis/index AND www.sciencemag.org/prizes/

Continued from page 1209

Ciccarelli *et al.* (p. 1283) used genomic information to construct a tree that can be easily automated and updated. They started with 36 genes universally present in 191 species for which orthologs could be unambiguously identified. An important component was a procedure for identifying and removing apparent lateral gene transfer effects. Using this open-source resource, the authors confirmed phylogenetic relationships and put forward hypotheses about the ancestor to modern bacteria.

MAPK Signaling 1: Development

Cardio-facio-cutaneous (CFC) syndrome is a rare disorder characterized by a distinctive facial appearance, skin abnormalities, heart defects, and growth delays. **Rodriguez-Viciana *et al.*** (p. 1287, published online 26 January) show that the disorder is caused by acquired mutations in genes encoding components of the mitogen-activated protein kinase (MAPK) signaling pathway. About 90% of the 23 patients studied carried missense mutations in the *BRAF*, *MEK1*, or *MEK2* genes that functionally altered the corresponding proteins. This discovery highlights the critical role of the MAPK pathway in human development and provides a tool for molecular diagnosis of CFC syndrome.



MAPK Signaling 2: Reversible Rescue

Chemical rescue of catalytically defective mutant enzymes has been a productive approach to studying enzyme function in vitro, but applications of the technique in vivo have so far met with limited success. **Qiao *et al.*** (p. 1293) have achieved rapid and reversible rescue of the protein tyrosine kinase Src in live cells using the small molecule imidazole. The work provides insight into the MAP kinase signaling pathway, including identifying several new Src substrates. Besides being a useful tool for studying cell signaling, small molecules that rescue disease-related mutant enzymes may have therapeutic potential.

Analog Axonal Signaling

Traditional accounts of intraneuronal electric signal transmission have distinguished between digital signals (action potentials) and analog (graded) signals. In mammals, analog signals are thought to occur only in primary sensory systems, like photoreceptors or bipolar cells. The brain has been thought to use digital action potentials to mediate dendritic input to the axon terminal. **Alle and Geiger** (p. 1290) suggest that this may be wrong: analog signaling is used by axons even in the middle of the brain. These recordings demonstrate passive transmission of dendritic potentials all the way up to the axonal terminal in a brain neuron and show the modulation of excitatory postsynaptic signals by analog presynaptic signals.

Do As You Would Be Done By

Lending assistance to relatives fits easily into evolutionary theory. Behaving in similar fashion with regard to unrelated individuals is harder to explain but undoubtedly occurs, at least amongst humans (see the Perspective by **Silk**). How, then, do you decide whether to cooperate with a potential partner? **Melis *et al.*** (p. 1297) asked whether cooperation is uniquely human. In two situations, they found that chimpanzees recruited a partner to help them to solve a difficult task and that they prefer partners who are more adept. **Warneken and Tomasello** (p. 1301) tested matched situations on human infants and young chimpanzees, in which subjects were given the opportunity to commit a helpful action without reward. Infants were quite ready to help a stranger with a task, such as stacking books in a pile or placing them onto a cabinet shelf, and chimpanzees also displayed to a limited degree a similar capacity for altruism.

CREDIT: RODRIGUEZ-VICIANA *ET AL.*

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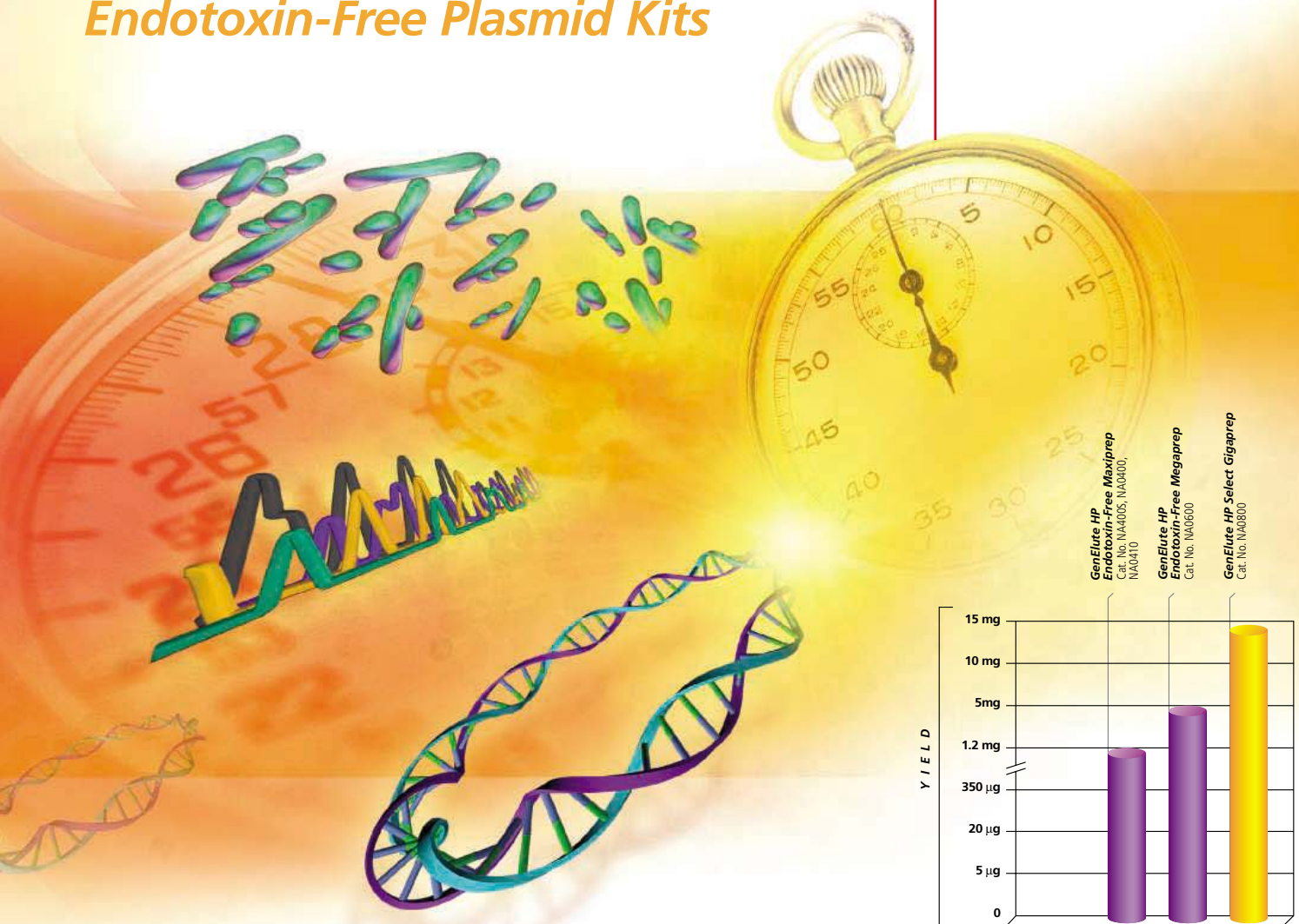

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The Mailbag

THE EDITOR'S DESK AT *SCIENCE* RECEIVES A SUBSTANTIAL FLOW OF COMMUNICATIONS. SOME OF THE arriving material consists of letters "sponsoring" important manuscripts or inquiring about the suitability of an attached manuscript for publication. These take time, but we are glad to spend it because we get some gems this way. Others are complaints: about the quality of already published papers (we advise the authors of these to contribute a Technical Comment); about the size of our fonts (these are mostly from people my age, so of course they're treated with exquisite sympathy); or from authors pointing out that our editors, the blind fools, have failed to see the scientific merit of their study.

There is a quite different category, which might belong under a heading called "author's remorse." These come in two subclasses: "Add me to the author list" or "Take me off." Wannabe authors of the first kind have a strong sense of having been left off the list unfairly; they cite the extent of their participation in the experiments and often hint darkly of personal animus on the part of the lead author. There is little we can do about these except to consult the listed authors and then, if necessary, turn the case over to the institution to sort out. As for getting off the list, just because there's bad news about the already-published paper, forget it. As they say in the pottery shop: "You broke it, you bought it."

I've had two experiences during the past 6 years that are quite different, in that I found myself urged by distinguished senior scientists *not* to publish a paper from another group that we were evaluating. This is a surprising departure from the prevailing idea in the scientific community that resolution through journal-mediated debate is preferred to censorship. The recent event involved a study by Donato *et al.* (*Science* Express, 4 January 2006) showing that salvage logging in a burned forest inhibited regeneration. The lead author is a graduate student in Forest Science at Oregon State University (OSU), and his coauthors include faculty colleagues in that department. We received a letter on 17 January 2006 signed by several senior OSU faculty members, mostly from the Department of Forest Engineering. It asked that we not publish the paper (apparently not appreciating the fact that its online posting amounted to publication). The letter contained arguments against the methods used in the Donato study.

This raised serious questions inside OSU. Should senior scientists attempt censorship of a paper from colleagues at the same institution? Faculty members in other departments and at other universities who were aware of the situation expressed deep concern about whether academic freedom was under threat at OSU. We told the letter-writers that we don't believe in censorship at *Science*, that it was too late to do what they asked even if we had been willing to, and that they could put their scientific objections in a Technical Comment.

But the issue didn't just disappear. The U.S. Bureau of Land Management (BLM), the source of funds for the study, quickly told OSU that it was withdrawing support for work by the Donato group. Fortunately, that lasted about 24 hours, after which the OSU administration took a firm stand on the matter. BLM promptly rescinded the action and restored funding. In other good news, the provost and the chair of the OSU Faculty Senate issued a strong statement in defense of academic freedom. The authors of the letter to *Science* may get some counseling about collegial behavior, which they surely need.

This brouhaha evoked some déjà vu. In 2002, we were considering a paper from investigators at Oak Ridge National Laboratory (ORNL) that provided evidence for nuclear fusion occurring in rapidly collapsing bubbles in deuterated acetone. ORNL management wanted some additional assurances from the investigators, and we delayed publication for a short time. But in came letters from two very senior physicists—one of them the leader of a large-scale fusion experiment—decrying the very notion of tabletop fusion and advising against publication. We went ahead anyhow. A confirming experiment with an improved design by some of the same authors has now appeared in *Physical Review Letters*. Of course, confirmation from an independent group is still welcome. But at least this question is up for resolution in the open literature, right where it belongs.

–Donald Kennedy

10.1126/science.1126280

A wasp's nest.

ECOLOGY/EVOLUTION

Asymmetric Nurture

An almost defining feature of the social hymenoptera (wasps, bees, and ants) is the absence of male workers; typically, females perform all of the tasks associated with care of the nest and larvae. Theoretical explanations centered on the genetic asymmetry of males and females (the males being haploid and the females diploid) have been discussed for decades, though experimental studies of this question have been few.

Sen and Gadagkar investigated whether males of the Indian wasp *Ropalidia marginata* would feed larvae, by manipulating the presence of females and the amount of food nearby. When food supplements were available and when females were missing, males were able to provision larvae at a frequency similar to that observed for females. It appears that under normal circumstances, males do not have enough access to food or are prevented from feeding larvae by females. Thus, the capacity to feed larvae is common to both sexes, and the mechanism preventing males from doing so may be behavioral rather than genetic or developmental. — AMS

Anim. Behav. **71**, 345 (2006).

GEOPHYSICS

There and Back Again

As waves produced by earthquakes reverberate through the solid Earth, they can be reflected or scattered from discontinuities within and between the mantle and core. Changes in the composition and temperature of mantle minerals can cause the waves to speed up, slow down, or bend and even reverse their paths. By monitoring earthquakes occurring within 10° of a seismic receiver array in Alaska, Tkalčić *et al.* have spotted a new phase of seismic pressure wave. These waves appeared to travel directly through the center of the Earth and inner core, and bounced back after scattering off the underside of a discontinuity in the upper mantle, 150 to 220 km below Antarctica. Because these waves were back-scattered just below the surface, they arrived at the receiver about a minute ahead of similar waves reflected from the antipodal surface itself; hence the authors termed them P′P′ near-podal precursors. The scatterers could be lenses of partially melted minerals or could comprise local concentrations of material different in composition than the rest of the upper mantle. — JB

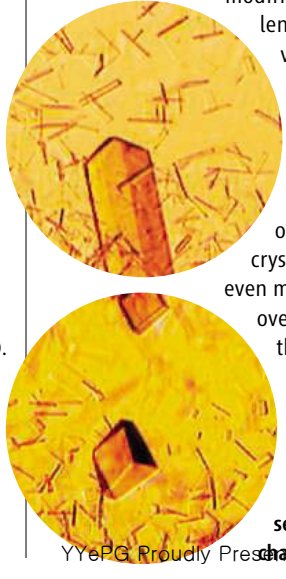
Geophys. Res. Lett. **33**, 10.1029/2005GL024626 (2006).

BIOTECHNOLOGY

Crystals on a Chip

Protein crystallization is a complex and often unpredictable process, which depends critically on buffer conditions and dehydration rates. Recently, microfluidic reactors have proven useful for screening a range of crystallization conditions with little material. However, these systems have rarely produced crystals large enough for analysis, nor has it been possible to preserve the crystals that do form for diffraction studies at cryogenic temperatures.

Hansen *et al.* have built a microfluidic device consisting of five parallel chambers, separated by a semipermeable membrane from a larger fluid reservoir. The osmotic strength of each chamber is equilibrated through internal diffusion among the chambers, as well as by a slow influx of vapor through the membrane. This motif can be repeated multiple times on a chip, with mixing times precisely controlled by modification of the channel lengths and chamber volumes. For lysozyme, ferritin, insulin, and catalase, they found that modulation of the mixing kinetics offered control over crystal quality, size, and even morphology. Moreover, crystals grown in these chambers could be preserved and studied in situ by x-ray diffraction to <2 Å resolution. — MSL



Bladelike (top) and rhomboidal (bottom) crystal morphologies, selected by varying chamber lengths.

J. Am. Chem. Soc. **128**, 10.1021/ja0576637 (2006).

MICROBIOLOGY

Adapting All Too Well

Human-specific pathogens, such as *Helicobacter pylori* and *Mycobacterium leprae*, exhibit geographic variation that is linked to that of their host. Gagneux *et al.* show that this is also true of *M. tuberculosis* and, intriguingly, that this variation may be linked to infection dynamics. First, by screening tuberculosis samples from people encompassing a range of geographical origins, the international collaboration found six major lineages with distinct global footprints. Then, by analyzing over a thousand isolates from five human populations in San Francisco, they found that most belonged to three of these lineages: roughly a quarter to the Indo-Oceanic (the most ancestral), a quarter to the East-Asian, and about half to the Euro-American. By looking at chains of transmission, they saw that the lineages differed in secondary case frequency, with the Euro-American being the most successful and with each lineage transmitting most efficiently within its original population. They suggest that lineages might be adapted to distinct human populations, as seems to be reflected in the efficacy of bacillus Calmette-Guérin vaccination, which could have implications for new tuberculosis control strategies (see Gessler *et al.*, Policy Forums, this issue, p. 1245). — CA

Proc. Natl. Acad. Sci. U.S.A. **103**, 2869 (2006).

CREDITS (TOP TO BOTTOM): RUCHIRASEN; HANSEN *ET AL.*, *J. AM. CHEM. SOC.* **128**, 10.1021/JA0576637 (2006)

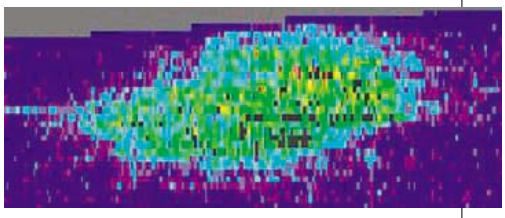
IMMUNOLOGY

Too Little or Too Much?

Crohn's disease is a severe inflammation of the mucosa of the intestine and is prevalent in developed countries. Multiple predisposing and environmental factors—such as mutations in the protein NOD2, which recognizes bacterial cell wall components—appear to influence the onset and progression of the condition, and current thinking is that these factors conspire to stir up unwanted immune reactions to the microflora of the gut.

Marks *et al.* provide evidence that Crohn's may instead be more representative of immunodeficiency. Crohn's patients were found to have reduced neutrophil accumulation and interleukin-8 (IL-8) production at sites of tissue trauma in the intestine and the skin. The defect in IL-8 production was independent of NOD2 mutation, and macrophages from patients were impaired in generating IL-8 in response to wound fluid from healthy individuals. Skin responses to subcutaneous injection of killed bacteria were also diminished, with local blood flow in the patients less enhanced relative to that in healthy controls. This is consistent with a lower potential for acute inflammatory responses in Crohn's patients; thus, although Crohn's disease may culminate in a chronic inflammatory response, it may originate in deficient acute pro-inflammatory responses to bacteria. — SJS

Lancet **367**, 668 (2006).



Increased forearm blood flow after injection of bacteria.

CHEMISTRY

Building a Staircase

Despite carbon's propensity to adopt a tetrahedral bonding geometry, chemists have managed over the years to squeeze it into a wide range of strained shapes, such as cubes and dodecahedra. However, it was remarkable to find that anaerobic *Candidatus* "Brocadia anammoxidans" bacteria, which are presumably more concerned with function than geometry, produce a fatty acid derivative in which the acyl chain is tethered to five cyclobutane rings, fused through shared edges as in a staircase. Despite an estimated strain energy of 75 kcal/mol, this molecule is a primary component of the intracellular membrane in which ammonia is metabolized.

Mascitti and Corey previously synthesized this compound in racemic form and have now achieved an efficient asymmetric synthesis, in which the C_8 carboxylate chain is bound to one specific external corner of the staircase motif. The authors achieved enantioselection through the use of a bulky dimethylphenylsilyl group, which directed cyclopentenone orientation in the photoinduced [2+2] cycloaddition that formed the fourth cyclobutane ring. In general, the synthesis relied heavily on cyclizations and rearrangements induced by ultraviolet irradiation. How the bacteria make this molecule (presumably in the dark) remains a mystery. — JSY

J. Am. Chem. Soc. **128**, 10.1021/ja058370g (2006).

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<< Mice Are Not Men

Pro-opiomelanocortin (POMC) undergoes posttranslational processing to yield a bunch of physiologically active peptides. In the hypothalamus, POMC is a precursor to the melanocortins (α-MSH, β-MSH, and γ-MSH). Humans and mice lacking functional POMC or MC4R (melanocortin-4 receptor, which is activated by α- and β-MSH) become obese; because rodents cannot synthesize β-MSH, this effect has been attributed to α-MSH. Biebermann *et al.* find that a severely obese child has a mutant form of β-MSH in which a cysteine has been substituted for a tyrosine, a mutation also present in obese family members. Restriction enzyme analysis of 722 obese and 1270 non-obese children and adolescents uncovered the mutation in 2 obese individuals and none of the controls. Lee *et al.* discovered the same β-MSH variant in 5 of 538 unrelated severely obese children and 1 of 300 non-obese adults and found that the mutation segregated with obesity in family members. Both groups observe that the mutant form showed substantially reduced binding to human MC4R and conclude that, unlike in rodents, β-MSH is important in regulating energy balance and body weight in humans. — EMA

Cell Metab. **3**, 141; 135 (2006).

CREDITS: MARKS *ET AL.*, *LANCET* **367**, 668 (2006)

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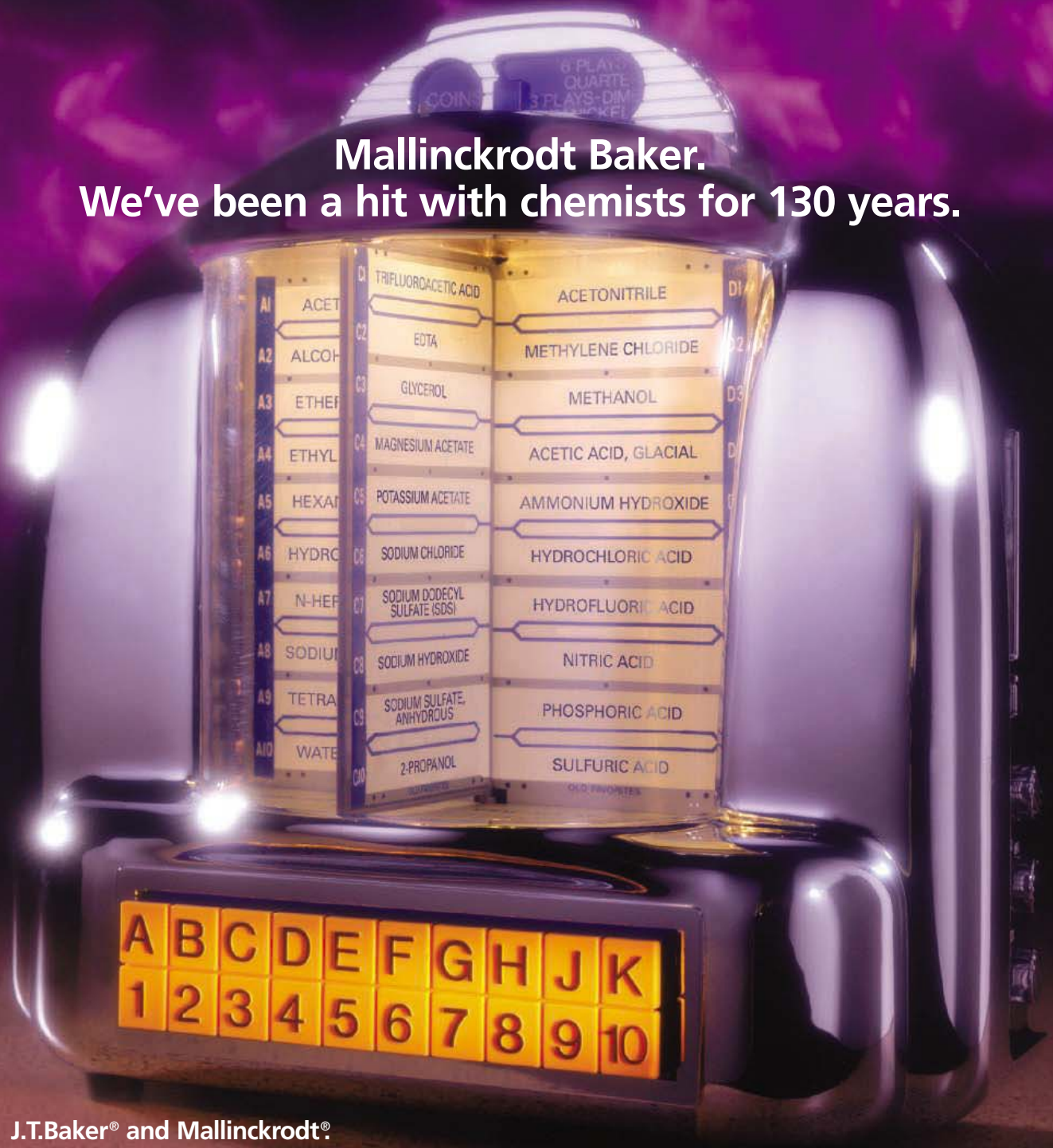

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EXHIBITS

Little (and Big) Engines That Couldn't

A bicycle powered by solid-fuel rockets sounds like one of Wile E. Coyote's schemes for catching the Road Runner. But in the 1920s and 1930s, German inventors built and even raced the souped-up cycles. In a 1931 trial, one model (above) reportedly hit 88 km/h before the "pilot" wiped out. The rocket bike is one of the doomed designs on display at the Museum of RetroTechnology, curated by London-based audio equipment designer Douglas Self. Crammed with period photos, the exhibits explore dubious achievements in transportation, power generation, computing, and communications. Self explains how the machines worked—most got at least to the prototype stage—and why they failed to catch on. Although it's tempting to laugh at contraptions like the strap-on helicopter and the steam lawnmower, "poking fun at misguided inventors is absolutely not the aim of the museum," Self says. Instead, he says, scrutinizing these machines might furnish insight into how inventors create. >> www.dself.dsl.pipex.com/MUSEUM/museum.htm

DATABASE

Flu Finder

Need to know which hemagglutinin proteins were carried last year by influenza viruses in Asia? Want to compare your viral samples to the deadly H5N1 subtype? Visit the Influenza Virus Resource from the U.S. National Center for Biotechnology Information, which houses all influenza virus sequences stashed in GenBank and provides tools for analyzing them. Users can dissect viral proteins and nucleotide sequences from all over the world and from a variety of hosts, including humans, pigs, and birds. >> www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html

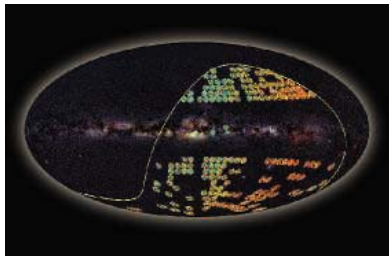
RESOURCES

The Compleat Evolutionist

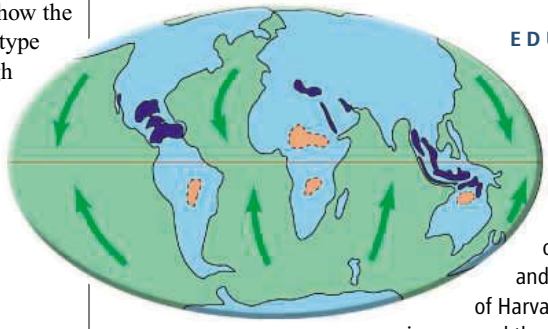
Charles Darwin recorded his experiments, observations, and thoughts in 16 books, 150 papers, and more than 80,000 pages of notes. This new digital library from the American Museum of Natural History in New York City will post the Darwin oeuvre, including previously unpublished notebooks and drafts, along with a host of other key evolutionary texts. Among the titles already on the shelves are two of Darwin's early sketches on natural selection and his colleague Thomas Huxley's book on human evolution. The library will add works by his predecessors, successors, and detractors, including early French anatomist Georges Cuvier, the late Stephen Jay Gould, and Edward O. Wilson. >> darwinlibrary.amnh.org

DATABASE

Stellar Speed Trap >>



A new database may help astronomers figure out how much our galaxy weighs and whether it filched some of its stars from other galaxies. The Radial Velocity Experiment is an international project to gauge the temperature, composition, surface gravity, and speed of up to 1 million Southern Hemisphere stars by 2010. Captured by an instrument at the Siding Spring Observatory in Australia, the data supplement other surveys, such as the Hipparcos mission, by adding hard-to-obtain readings of radial velocity, a star's speed toward or away from us. Last month, the site posted the first measurements on nearly 25,000 stars. This view of the night sky (above) shows the project's current coverage, with red marking the swiftest stars. >> www.rave-survey.aip.de/rave



EDUCATION

The Really Big Chill

If the "snowball Earth" hypothesis is right, our planet froze at least three times in the distant past. To learn more about the controversial notion, schuss over to this site sponsored by geologist and snowball Earth advocate Paul Hoffman of Harvard University. The hypothesis holds that ice encased the planet for several million years starting about 2.2 billion years ago, again 710 million years ago, and then 640 million years ago. Background pages present supporting evidence and probe the cold spells' possible causes and consequences for life. One trigger may have been the continents clumping along the equator, where the torrid conditions could have paradoxically set off a global chilling by accelerating a form of weathering that depletes atmospheric carbon dioxide. The site also offers nearly 200 downloadable slides for classroom use, such as this map of "oases" where life might have endured the big freezes (the orange and blue blotches above), and other resources. >> www.snowballearth.org

Send site suggestions to >> netwatch@aaas.org

Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): HULTON ARCHIVE/GETTY IMAGES; THE RAVE COLLABORATION; PAUL HOFFMAN/HARVARD UNIVERSITY

Meave Leakey with Kenyan grad student Fredrick Kyalo Manthi.

DESERT OUTPOSTS

Famous fossil hunters Richard and Meave Leakey have joined forces with Stony Brook University in New York to build a research institute in the remote desert of Lake Turkana in northern Kenya.

The Leakeys and their colleagues have unearthed a stunning series of fossils of early human ancestors at the lake over the past 40 years. Now they aim to set up a modern facility comprising at least two year-round field stations that will serve as a staging ground for fieldwork in the vast badlands around the lake, where fossils date as far back as 65 million years.

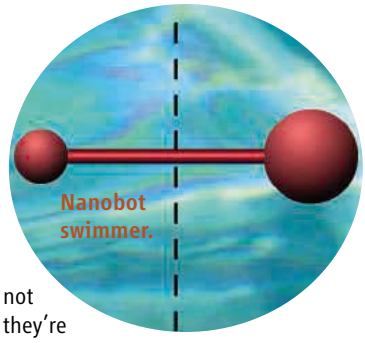
With a permanent institute, "we could triple the amount of time spent in the field and establish an international educational outreach program through satellite links," says Richard Leakey, a visiting professor of anthropology at Stony Brook since 2002. Another goal is to train and hire African postdoctoral researchers and graduate students. Leakey has raised $1.5 million toward a $20 million goal from three wealthy donors, including Mexican telecommunications mogul Carlos Slim. The university has pledged so far to hire two new faculty members.

Glass With an Impact

In December 1932, scientists surveying the southern Egyptian desert came upon pieces of a translucent, pale yellow-green, glassy substance, from tiny fragments to football-sized chunks, scattered over a huge area at the Libyan border. Known as Libyan desert glass, this almost pure silica contained isotopes showing it to be of extraterrestrial origin. But scientists haven't been able to figure out where it came from.

Now Farouk El-Baz, director of the Boston University Center for Remote Sensing, believes the mystery has been solved. This month, poring over satellite images of the Sahara Desert, he found a gigantic impact crater in the area. At a diameter of 30 kilometers, it's "the largest crater yet found in the Sahara," El-Baz says, and big enough to be the source of the glass, which covers a 60- by 100-kilometer area. He believes the crater hadn't been recognized before because it is so big; also, parts of its rims were eroded by two ancient river systems. El-Baz has named the crater, located on the Gilf Kebir plateau, the Kebira. "This is a large crater and well worth scientific investigation," says Friedrich Horz, a crater expert at the Johnson Space Center in Houston, Texas.

TAKE A DIP IN THE FERMI SEA



Recipe for the ultimate extreme winter sport: Set nanometer-sized robots swimming in a pool chilled to near absolute zero.

Nanobot swim sprints might not make the Olympics, but in theory they're possible, say mathematical physicists. Joseph Avron, Boris Gutkin, and a colleague at the Technion–Israel Institute of Technology in Haifa, Israel, had previously studied larger robots swimming in a viscous fluid and decided to see what would happen at the nanoscale. They imagined robots consisting of spheres and rods capable of changing sizes and lengths in rhythmic patterns, in a rough analogy to swimming strokes, immersed in a supercold fluid of particles called fermions, which are described by quantum waves. Wriggling in certain ways, the robots transmit waves of fermions in one direction, pushing themselves in the other. With each "stroke," a swimmer moves a distance equal to a multiple of half the typical wavelength of the fermions, the researchers will report in an upcoming issue of *Physical Review Letters*. The swimmer also can move without losing energy.

The analysis may not be practical, but it was conceptually appealing, Avron says: "This is the kind of license a theorist can have." Leonid Levitov, a theoretical physicist at the Massachusetts Institute of Technology in Cambridge, agrees: "[The result] being beautiful is reason enough for doing the work."

ROCKIN' TO THE MUSIC GENOME

If a band member is like an organ, contributing to the functioning of the whole body, what are the different sounds the band produces? Tim Westergren calls them genes. He's the brain behind the Music Genome Project, designed to "capture the details that collectively describe a piece of music, the same way the genome does for a person."

Westergren and some 30 fellow music enthusiasts run a company called Pandora, which analyzes songs according to features, or "genes," such as instrumentation, lyrics, beat, mood, and type of harmony. So far, they've cataloged about 400 genes, each with different forms: Voice, for instance, has 30 different "alleles," from urban to sultry. When a visitor to the Web site (www.pandora.com) enters the name of a song, an algorithm runs through all the genomes in the database and creates a playlist of "relatives."

The project is "a cute strategy" for analyzing music, says genomicist Elliott Margulies of the National Human Genome Research Institute (who is also the keyboard player in a rock band). "It's like looking at human variation or primate evolution; they're trying to analyze the same genes to look at the variation within music."

Westergren says users are sometimes surprised: They'll input a favorite song—say, a mellow Sarah McLachlan tune—and Pandora will come back with a pop hit by Britney Spears. Some people don't like being reminded that humans are related to monkeys either, but the genes don't lie.

CREDITS (TOP TO BOTTOM): ANN GIBBONS; LANDSAT; AVRON *ET AL.*, *NEW J. PHYS.*, **7** 234 (2005)

Genomes to Life
1226

A counter-protest
1229

Showing her cards. Ilaria Capua says she will submit H5N1 sequences from her lab to public databases immediately.

AVIAN INFLUENZA

As H5N1 Keeps Spreading, A Call to Release More Data

PARIS—An impassioned call by a prominent Italian influenza scientist has renewed the debate about how to balance global health against scientists' needs to publish and countries' demands for secrecy. On 16 February, Ilaria Capua of the Istituto Zooprofilattico Sperimentale delle Venezie in Italy asked more than 50 colleagues around the world to release all sequence data for the H5N1 avian influenza strain into the public domain. Comparing sequence data from every H5N1 isolate as soon as they become available is crucial for understanding how the virus moves and evolves, Capua argues.

Putting her money where her mouth is, Capua entered H5N1 sequence data from two recently infected countries, Nigeria and Italy, into the GenBank database the same day. She also rejected an offer by the World Health Organization (WHO) to join a select circle of 15 labs that share bird flu sequences on a password-protected Web site.

Capua's lab is a reference center for the U.N. Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (OIE), and officials at those agencies say they support her call. But some scientists say sharing data instantly is complicated by the need for credit, and WHO argues that without some form of confidentiality, some countries would not submit samples at all.

Sharing information about H5N1 has been tricky from the start. WHO, FAO, and OIE encourage countries to send virus samples to specialized reference labs that can confirm the outbreak and study the virus further. Some have been reluctant to do so because they worry about intellectual-property rights or not receiving a fair share of the scientific credit; China, for instance, has not shared any avian samples for a year, a WHO spokesperson says. But even when reference labs do get their hands on a virus, they don't always release the data immediately.

For instance, in the past few months, H5N1 samples from about 15 European countries have been sent to the Veterinary Laboratories Agency (VLA) in Weybridge, U.K., a reference lab for OIE and the European Union. Lab director Ian Brown says he's sharing sequence and other data with governments and the international agencies; to show support for Capua's campaign, he also submitted the sequence of a virus from an outbreak in Turkey that he says is a "progenitor to the European epidemic" into GenBank last week. However, until a paper about the European outbreaks—which he says could be submitted in a matter of weeks—has been accepted, Brown says he needs to hold on to the European sequences. "The staff in this institute is working 24/7 to provide this service," he says. "I don't think it's unreasonable to expect … some reward for their endeavors." It also takes time to negotiate the conditions for releasing data with dozens of individual governments, Brown says.

Capua counters that just isolating and sequencing a virus that comes in the mail does not give researchers the right to sit on the data—especially not at a government lab. "Most of us are paid to protect human and animal health," she says. "If publishing one more paper becomes more important, we have our priorities messed up." Governments can often be persuaded to release the sequences, adds Capua, who repeated her call at an OIE meeting in Paris on Monday and also plans to submit it to ProMED, an e-mail list about emerging infectious diseases.

WHO agrees that in an ideal world, scientists would share their data widely and voluntarily, says Wenqing Zhang of the agency's Global Influenza Programme. But because that's not happening, the agency created a special secured section at the Influenza Sequence Database at Los Alamos National Laboratory in New Mexico in 2004. Currently, some 15 labs have passwords to access these data, says Zhang, including WHO's eight reference labs. The system is invaluable for WHO, she adds, as it helps the agency track the virus and adjust risk assessments if necessary.

Virologist Yi Guan of the University of Hong Kong, which has a huge H5N1 collection, says he would be prepared to release more data publicly before publication but is looking for WHO to establish a new policy. Until then, WHO's secure server at least ensures that policymakers and most of the scientists who advise them have access to vital information. But Capua says everyone with an interest should be able to browse all the data. When she was offered access in exchange for submitting her Nigerian sequence last month, she declined. And the system gets mixed marks within WHO as well. "Personally, I'm not in favor of it," says WHO scientist Michael Perdue.

Whether scientists' fears of being scooped are justified is difficult to say. In theory, once sequences are posted in the public domain, anybody could write a paper about them. In practice, journal editors will ask manuscript authors to get permission if they write a paper about unpublished data they did not submit to GenBank themselves, says Caroline Ash, who edits infectious diseases papers at *Science*. But Brown says he'd rather not take that risk.

–MARTIN ENSERINK

With reporting by Dennis Normile in Tokyo.

CREDIT: ANNA FOGAROLO/CLICK FOTO AGENCY

AVIAN INFLUENZA

Evidence Points to Migratory Birds in H5N1 Spread

With the H5N1 avian influenza virus racing across the globe, scientists are debating new evidence on the role of migratory birds. As *Science* went to press, the virus had just been confirmed in a third African nation, Niger, one of the world's poorest countries. It had spread further in Europe and Asia, with 13 countries confirming outbreaks in just the past 2 months. And France reported the European Union's first outbreak in domestic poultry.

Increasingly, scientists are attributing this remarkably fast spread to migratory birds, but dissenters remain. One set of data that points to a role for wild birds comes from recent, unpublished analyses of influenza viruses recovered from outbreaks stretching from Russia and Kazakhstan to Nigeria, Iraq, and Turkey. A World Health Organization report issued last week,* which drew upon these analyses, concluded that all of the viruses involved in these outbreaks appear to be related to the strain identified from Qinghai Lake in northwestern China, where an outbreak killed 6000 wild birds last spring. And instead of the constant evolution typical of avian viruses, the Qinghai variant appears to have remained unusually stable for nearly a year. "This finding raises the possibility that the virus—in its highly pathogenic form—has now adapted to at least some species of migratory waterfowl and is … traveling with these birds along their migratory routes," the WHO report concludes.

That case is strengthened by the first documented identification of the H5N1 virus in healthy migratory birds, reported in the 21 February issue of the *Proceedings of the National Academy of Sciences* (*PNAS*). Some researchers have expressed skepticism that migratory birds play a major role in the spread of H5N1, arguing that infected birds would die before traveling very far (*Science*, 21 October 2005, p. 426). The new findings, from a collaboration led by Yi Guan, a virologist at the University of Hong Kong, and virologist Robert Webster of St. Jude Children's Research Hospital in Memphis, Tennessee, suggest that's not always the case. Since early 2003, the team has collected more than 13,000 cloacal and fecal samples from migratory birds at Mai Po Marshes in Hong Kong and Poyang Lake in Jiangxi Province, China. In early 2005, they isolated the H5N1 virus from six apparently healthy migratory ducks at Poyang Lake. The team also collected serologic samples from 1092 captured migratory ducks and found that 3.1% had antibodies to H5N1, indicating a prior infection.

The group's findings confirm that wild birds can carry the virus great distances. Their sequencing analyses show that the viruses isolated from Qinghai Lake are genetically linked to the two strains recovered from the wild ducks at Poyang Lake. Guan says this doesn't mean ducks from Poyang carried the virus to Qinghai but does suggest that these viruses are circulating among migratory birds.

Guan and his colleagues also have data suggesting that once an outbreak is established, the main route of transmission appears to be through poultry. The group has regularly sampled poultry brought to markets in six provinces in southeastern China since 2000. Among the more than 51,000 birds studied, they found the virus in 1.8% of all ducks and 1.9% of all geese, as well as 0.26% of chickens. Sequencing of 121 influenza samples collected from birds in China, Indonesia, Malaysia, and Vietnam showed that the viruses fall into regional sublineages. Viruses recovered from wild ducks at China's Poyang Lake were related to two sublineages from different regions in southern China. Guan says that together, this suggests that the viruses have been endemic among ducks and geese in different regions long enough to evolve distinct phylogenetic signatures and that circulation among poultry, not reintroduction from wild birds, is keeping the virus going in China. If migratory birds had repeatedly seeded the outbreaks, there would likely be fewer distinct regional differences in the viruses. Guan adds that this conclusion offers hope that the cycle of transmission can be broken if the virus is eradicated from poultry flocks.

Investigation. A veterinarian looks for signs of bird flu infection in a swan, found dead earlier in the day, at a lab in Arras in northern France on 22 February.

CREDIT: PASCAL ROSSIGNOL/REUTERS

The WHO report and *PNAS* study don't convince everyone that wild birds explain H5N1's alarming spread. "There is no single bird species that migrates due west-east," notes Richard Thomas, a spokesperson for Birdlife International. Guan counters that the spread could involve a complex interaction of humans transporting poultry and the movements of dozens of species of wild birds. "It is not easy to trace this step by step," he says.

The difficulty is seen in Europe, where dead swans symbolize the spread of the virus. Because they obviously succumb to the virus, no one thinks swans are carrying it great distances. "Swans become infected by other aquatic [bird] species," says Albert Osterhaus, a virologist at Erasmus University Medical Center in Rotterdam, the Netherlands. But he admits that as yet, surveillance efforts in Europe have not found H5N1 in any healthy wild birds. "We do not, at this moment, have the complete epidemiological picture," Osterhaus says. He adds that more surveillance of wild birds is needed along with lab experiments to study the behavior of the virus in different migratory species.

–DENNIS NORMILE

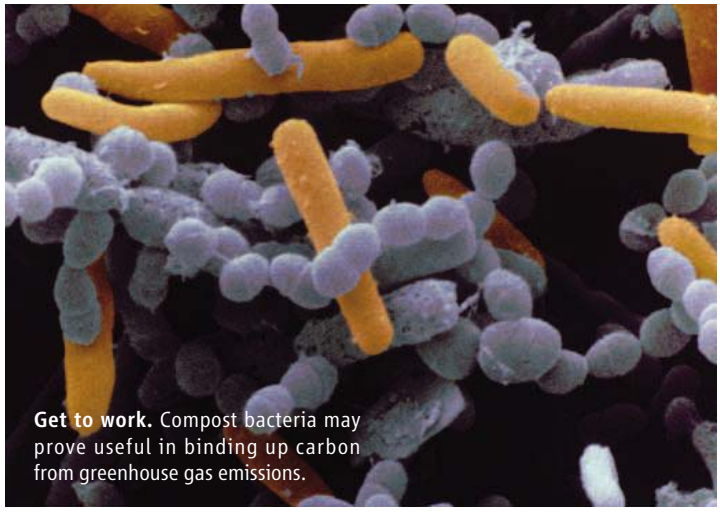
* www.who.int/csr/2006_02_20/en/index.html

GENOMICS

DOE Hits Potholes on the Road to Systems Biology

The Department of Energy's bold plans to expand its genomics efforts drew some critical comments from a panel of the National Research Council (NRC) last week. The panel would like DOE to spend more money and take a different approach.*

The $70-million-a-year Genomes to Life Program, begun in 2000, has led the way in sequencing microbes involved in bioremediation, carbon sequestration, and bioenergy, as well as in deciphering genomes of other key organisms. Late last year, DOE announced that the next phase of the program, renamed Genomics:GTL, would focus on systems biology, and last month President George W. Bush requested $119 million in 2007 for those efforts. DOE plans to fund four centers, each with a different technological bent: large-scale characterization of proteins, imaging complex molecules, proteomics, and systems biology. Each center would serve academic and corporate scientists. DOE plans to build these centers over the next 2 decades and has already invited proposals for the protein-production facility.



Get to work. Compost bacteria may prove useful in binding up carbon from greenhouse gas emissions.

Before DOE goes forward, however, the NRC panel wants each center to focus on a specific problem, say, bioenergy or bioremediation, and encourage scientists from all relevant disciplines to lend a hand. Such "one-stop shopping … is a change from DOE's more typical historical model of providing just a user facility, like the synchrotron," says Jennie Hunter-Cevera, a microbial physiologist and president of the University of Maryland Biotechnology Institute in Rockville. But she and others say focusing the centers on problems rather than technologies would encourage more interaction among researchers.

The NRC panel argued for a tripling of the program's current annual budget, to as much as $200 million, but it also suggested ways to cut costs, get the centers up and running more quickly, and increase interaction with outside researchers. One solution: Occupy empty space in an existing biotechnology corridor and ask public and private institutions to foot the bill for renovations or construction. The committee urged DOE not to locate the centers at its 16 national labs because security at the labs might limit access.

Program managers for Genomics:GTL say they need more time to review the recommendations. But Betty Mansfield, a biologist at Oak Ridge National Laboratory in Tennessee who was involved in the early planning for the genomics program, worries that the panel's suggestions won't save money. She says that DOE rejected the idea of having centers focus on particular problems because "you end up with redundant technology. And with that redundancy comes increased costs." **–ELIZABETH PENNISI**

* *Review of the Department of Energy's Genomics:GTL Program* (fermat.nap.edu/catalog/11581.html)

SCIENTIFIC PUBLISHING

Canadian Editors Fired in Row With Association

TORONTO—The editor of Canada's premier medical research journal and a top assistant have lost their jobs after a long-running feud with the publisher over editorial independence.

John Hoey, editor of the *Canadian Medical Association Journal* (*CMAJ*), and Senior Deputy Editor Anne Marie Todkill were dismissed without notice last week by CMA officials. Graham Morris, head of CMA's media division, says, "I felt it was time for a fresh approach." Morris claims the journal's independence was not an issue but adds, "The last call will be my call" in any dispute over content. This week, the Council of Science Editors condemned CMA's action. CSE President Richard Horton, editor of *The Lancet*, called it "a blatant example of the misuse of power, in promoting an agenda that goes beyond the legitimate authority of the journal's owners."

The dismissals came after a series of clashes between Hoey and his bosses. In November 2002, the journal ran a letter from 20 members of the journal's editorial board saying that then–CMA President Dana Hanson posed a "clear and present danger" to the journal's editorial independence after Hanson had demanded Hoey retract an already-published editorial on medical legislation. Two months ago, Hoey described in an editorial how CMA officials had ordered him to revise an unpublished investigative article on questions Canadian women were being asked when trying to buy the nonprescription emergency contraceptive Plan B after the Canadian Pharmacists Association complained about the investigation.

Hoey then called in Jerome Kassirer, who was forced to retire in 1999 as editor of *The New England Journal of Medicine* amid a similar debate over editorial independence with its publisher. Kassirer says he believes CMA violated guidelines from the International Committee of Medical Journal Editors that publishers "should not interfere in the evaluation, selection, or editing of individual articles" and that editors are obliged to speak out. "It's my belief the Canadian Medical Association has commandeered the journal," says Kassirer, who as a *CMAJ* board member signed the November 2002 letter.

Another signer, Donald Redelmeier of the Institute for Clinical Evaluative Sciences in Toronto, says that Morris "expressed no concerns" about Hoey at a board meeting last fall and that "customarily, we organize more tranquil succession timing." He and others worry that the firings could affect the flow of submissions to the journal. "We knew there was a fearful row going on," says Drummond Rennie, a deputy editor of *The Journal of the American Medical Association*. "There is no quicker way of destroying the reputation of a medical journal than suddenly firing the editor."

–PAUL WEBSTER

Paul Webster is a freelance writer based in Toronto.

CREDIT: SIMKO/VISUALS UNLIMITED

UNIVERSITIES

Despite a Chilly Reception, the 'European MIT' Advances

CAMBRIDGE, U.K.—Facing down skeptics in the academic community, European Union (E.U.) officials are forging ahead with a proposal to create a new research-intensive university on the continent. They say their objective is to remedy problems in European higher education by building a flagship modeled on the Massachusetts Institute of Technology (MIT). Rather than a single site, however, a plan published last week by the European Commission, the E.U.'s executive, calls for a network of centers across the 25 member states. But the idea continues to meet with near-universal hostility from scientific and education leaders. The commission "has failed to analyze what the issue is and how you would address it," says glaciologist Geoffrey Boulton of the University of Edinburgh, U.K., who has studied the plans for the League of European Research Universities (LERU).

Academics argue that there is no need for a new European Institute of Technology (EIT). "There are a lot of very good institutions [in Europe] that are grossly starved of funds," says Peter Cotgreave of the Campaign for Science and Engineering in the U.K., a pressure group. And they worry that the commission's new enthusiasm will attract attention—and funding—away from the new European Research Council (ERC), due to begin work next year. With the E.U. research budget still undecided, "we could take our eyes off a rather crucial ball," says Boulton.

CREDIT: HAZIR REKA/REUTERS

Flying the flagship. European Commission President José Manuel Barroso is a strong supporter of the European Institute of Technology.

Planners dreamed up the EIT early last year as part of the Lisbon Strategy, a faltering scheme to make Europe the leading knowledge economy by 2010. After a public consultation in the fall, the commission's outline last week argues that "Europe still falls short in turning R&D results into commercial opportunities." According to commission president José Manuel Barroso, who has championed the idea, "Excellence needs flagships; that's why Europe must have a strong European Institute of Technology."

The commission proposes a small governing board that would identify worthy areas of interdisciplinary research and set up "knowledge communities." These would borrow staff, students, and facilities from universities, research centers, and industrial labs across the E.U. for as long as 15 years. The EIT will, the commission asserts, be a high-quality "brand," and institutions will compete to join. E.U. heads of government will discuss the plan at the end of March and, if they give it the nod, commission officials will draw up the legal documents. The EIT could be recruiting academic staff by 2009.

Although the attention on higher education is welcome, many dispute the idea that MIT's success can simply be transplanted onto European soil. "MIT is just a very good university, and many European universities are very successful in the same areas," says Boulton. Funding is another concern. The commission says the EIT will be funded by the E.U., national governments, and industry, and that not much will be needed before the end of the decade. But E.U. finances are already squeezed; the research budget—currently being debated by the commission and the European Parliament—will fall short of last spring's request.

One concern is the potential impact on funding for the ERC, a new grants agency. Unlike the E.U.'s Framework Programme, the ERC will have an independent scientific council and make awards based primarily on scientific excellence. "Although its funding is small, within a decade the ERC could be a very fundamental driver of research in Europe," says Boulton. "The ERC is a genuinely bottom-up proposal, something that's been debated and developed over 3 or 4 years," says John Smith, deputy secretary general for research at the European University Association. Adds LERU Secretary-General David Livesey: "Everyone agrees the ERC is the right thing to do at the moment. That's the flagship."

—DANIEL CLERY

Identity Crisis

It's back to the drawing board for biodiversity experts hoping to share data on the world's flora and fauna with policymakers. Plans for impartial assessments for international environmental conventions failed to gel last week in Paris, where there was "a lot of doubt about how to achieve this best," says Peter Raven, president of the Missouri Botanical Garden in St. Louis. Researchers, government officials, and conservationists hope over the next 18 months to develop what they are calling an International Mechanism of Scientific Expertise on Biodiversity that would have more political clout than the 1995 Global Biodiversity Assessment or the 2005 Millennium Ecosystem Assessment (*Science*, 1 April 2005, p. 41).

—ELIZABETH PENNISI

New Dover Board to Pay $1 Million

The Dover, Pennsylvania, school board has agreed to pay $1 million toward plaintiffs' costs in the suit on intelligent design (ID) it lost in December (*Science*, 6 January, p. 34). Judge John E. Jones III ruled that reasonable court costs totaled more than $2 million, but after 2 months of negotiations, lawyers for the winning side agreed to settle for less than half that amount. "We'll find a way to take care of it," says board member Bernadette Reinking. Eight board members who supported ID were voted out last fall, putting the onus on the new board to pay for the suit.

—CONSTANCE HOLDEN

Disease Alert Network Proposed

The head of Google's new foundation has begun his own philanthropic Internet project. Larry Brilliant, a physician and public health advocate, wants to improve a Canada-based network that scours the Web for early signs of disease outbreaks such as bird flu. Brilliant will seed his initial $10 million campaign with a $100,000 prize he received last week from a New York City–based group called Technology Entertainment Design for past work such as helping to eradicate smallpox and treat blindness in developing countries.

Public health experts applaud Brilliant's plans to troll millions of Web sites and publish free public disease alerts in dozens of languages. "Almost any initiative to identify infectious disease outbreaks would be welcomed by WHO," says World Health Organization spokesperson Maria Cheng.

—JOCELYN KAISER

SCIENCE INDICATORS

NSF Presents the Wide World of Science

China has arrived as a scientific powerhouse. Or has it?

The factors behind China's rapid rise to third place in overall research spending, behind only the United States and Japan, are documented in the latest compendium of international trends in science issued last week by the U.S. National Science Foundation (NSF). Its biennial *Science and Engineering Indicators* (nsf.gov/statistics/seind06) features analysis, statistics, and tables on everything from academic research spending to zoo attendance. As always, the two volumes are a gold mine of information. But the 2006 edition also comes with a refreshingly frank caveat.

One essay, for example, points to the danger of comparing research expenditures around the world and raises questions about one common metric called purchasing power parity (PPP). "It is difficult or impossible to assess the quality of PPPs for some countries, most notably China," it notes. "Although PPP estimates for [industrialized] countries are quite reliable, PPP estimates for developing countries are often rough approximations." In particular, China's R&D expenditures, reported at $84 billion in 2003, could be inflated by a factor of 4 or 5, it adds.

Another essay, on "unmeasured R&D," reminds readers that some sectors—businesses with fewer than five employees, for example—go unreported. Others, notably research done by nonprofit organizations and state and local governments, are extrapolated from surveys nearly a decade old.

A companion piece to the indicators report (nsf.gov/statistics/nsb0602) by the National Science Board, NSF's presidentially appointed oversight body, offers several suggestions for improving U.S. science and math education. It says higher pay for teachers, improved public literacy, and tests that measure both conceptual knowledge and problem-solving skills are needed to tackle what it calls "America's pressing challenge." The science board is also weighing launch of a commission that would examine the subject. **–JEFFREY MERVIS**

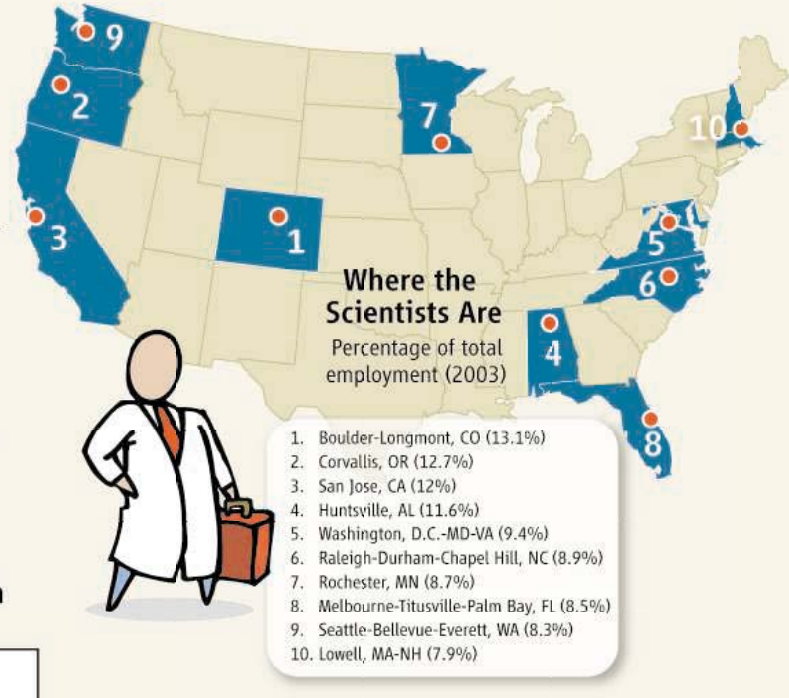
Did You Know ...

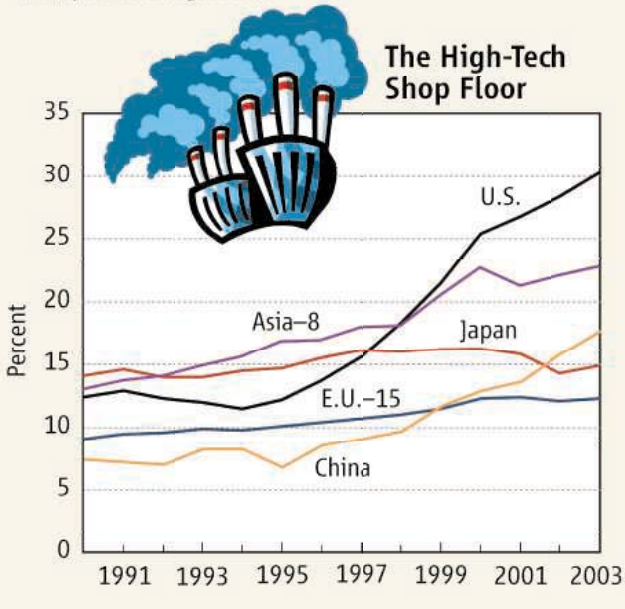
This year's *Science and Engineering Indicators* includes the following intriguing bits of information (clockwise from top right):

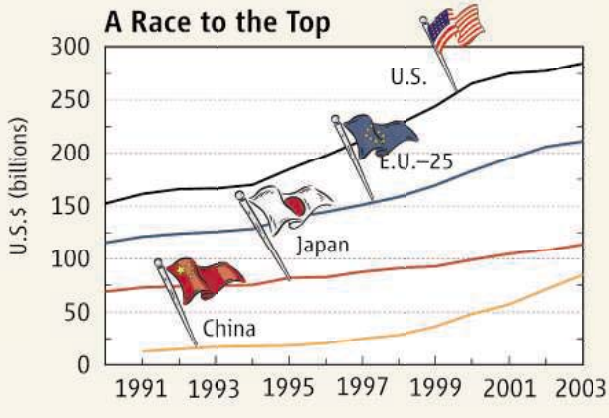
Meet market. Singles looking for scientifically inclined mates can be bolder in Boulder, Colorado, which boasts the highest concentration of scientific workers in its labor market. For sheer numbers, however, the Washington, D.C., area leads the way, with a quarter-million S&E workers.

Spending spree. China is moving closer to Japan on overall research spending but has a long way to go to catch the 25-nation European Union and the United States.

High-end goods. The United States and China have more than doubled the share of their manufacturing sector derived from high-tech products since 1990, while the situation in Europe and Japan has changed little.







SOURCE: *SCIENCE AND ENGINEERING INDICATORS 2006*

SCIENCE AND DIPLOMACY

Indian Chemist Receives a Visa and an Apology

NEW DELHI—In an abrupt turnaround, the United States last week "home delivered" a visa to Goverdhan Mehta, former director of the Indian Institute of Science in Bangalore, after holding up his application and questioning him about the potential use of his research in chemical weapons. The case raised concern in the scientific community. U.S. officials apparently hoped to smooth ruffled feathers before President George W. Bush's visit to India this week.

Offended. Goverdhan Mehta.

But Mehta is not mollified. His response to the U.S. offer: "Thank you, but no thank you. I have already canceled my tickets and have no intention of going to the United States," he told *Science*, which first reported the incident (*Science*, 17 February, p. 933). "I am not allergic to the United States and would be willing to go at a later date."

The U.S. Embassy issued a statement on 24 February saying that the ambassador to India, David C. Mulford, "called Professor Mehta … to notify him and express both his apologies and satisfaction that a visa would be issued immediately." The processing of the visa had been suspended pending a review in Washington, D.C., the embassy said, but it was later approved. Mehta says that when he first applied in early February, the consular office in Chennai questioned him and suggested that his research could be used in chemical warfare, then turned him away. It was the "most humiliating experience" in his life, Mehta says. A consular agent came to Mehta's laboratory on 24 February and collected the passport, which was delivered to the lab on Saturday with a visa stamp.

Another scientist who was recently turned down for a visa, Placid Rodriguez, former director of the Indira Gandhi Center for Atomic Research in Kalpakkam, also received a U.S. entry visa on 24 February in what he describes as a "huge turnaround." He feels that "all's well that ends well."

Mehta says, "I appreciate the apology extended by the U.S. ambassador." But he remains concerned: Scientists must be able to participate "in international activities without being subject to any such restriction or humiliation." **–PALLAVA BAGLA**

ANIMAL RESEARCH

Protesters March to a Different Drummer

OXFORD, U.K.—A placard-waving crowd took to the streets here on 25 February with an unusual message: Support animal research. Several hundred people showed up, among them a few speakers from the University of Oxford faculty, including neurosurgeon Tipu Aziz (surrounded by a crowd, right). The idea for the rally came from 16-year-old Laurie Pycroft, who describes himself as an Internet blogger and fan of science. Angered by an encounter in January with protesters seeking to halt construction of Oxford's $34 million life sciences lab, Pycroft decided to respond with a pro-lab march. The idea caught on. The same day, opponents of the lab staged a rally several blocks away; police kept them apart. Oxford has been the main target of animal-rights protests since the University of Cambridge gave up on plans for a primate facility 2 years ago. Last fall, the Animal Liberation Front took credit for torching an Oxford boathouse (*Science*, 5 August 2005, p. 872); ALF recently declared on its Web site that anyone connected to the university is "a legitimate target." **–ELIOT MARSHALL**

YePG Protest Planned Next Week for Support

CREDITS (TOP TO BOTTOM): P. BAGLA/*SCIENCE*; E. MARSHALL/*SCIENCE*

A Bid for Science Tourism

LONDON—Stem cell scientists should not be penalized for doing research in foreign countries with more permissive laws, states a new set of ethical principles for international scientific collaboration. The document, drawn up by the newly formed Hinxton Group, implicitly targets Germany, where most researchers are government employees and therefore could face jail time if they don't follow German laws on research when working abroad. The group's 24 February consensus statement (www.hopkinsmedicine.org/bioethics) includes guidelines for researchers and scientific journals. The group includes 60 scientists, lawyers, ethicists, and journal editors from 14 countries.

–MICHAEL SCHIRBER

Biotech: UC Milks It

In one of the largest biotechnology patent settlements ever, Monsanto Co. agreed this week to pay the University of California more than $100 million to settle claims that the agribusiness giant infringed on a patent awarded to UC researchers in 2004 for a hormone that makes cows produce more milk. Use of the hormone, called bovine somatotropin (BST), has spawned a $1 billion industry and drawn criticism from some consumer groups worried about health effects. UC says most of the royalties will support health and clinical research at UC San Francisco, where BST was discovered in 1979.

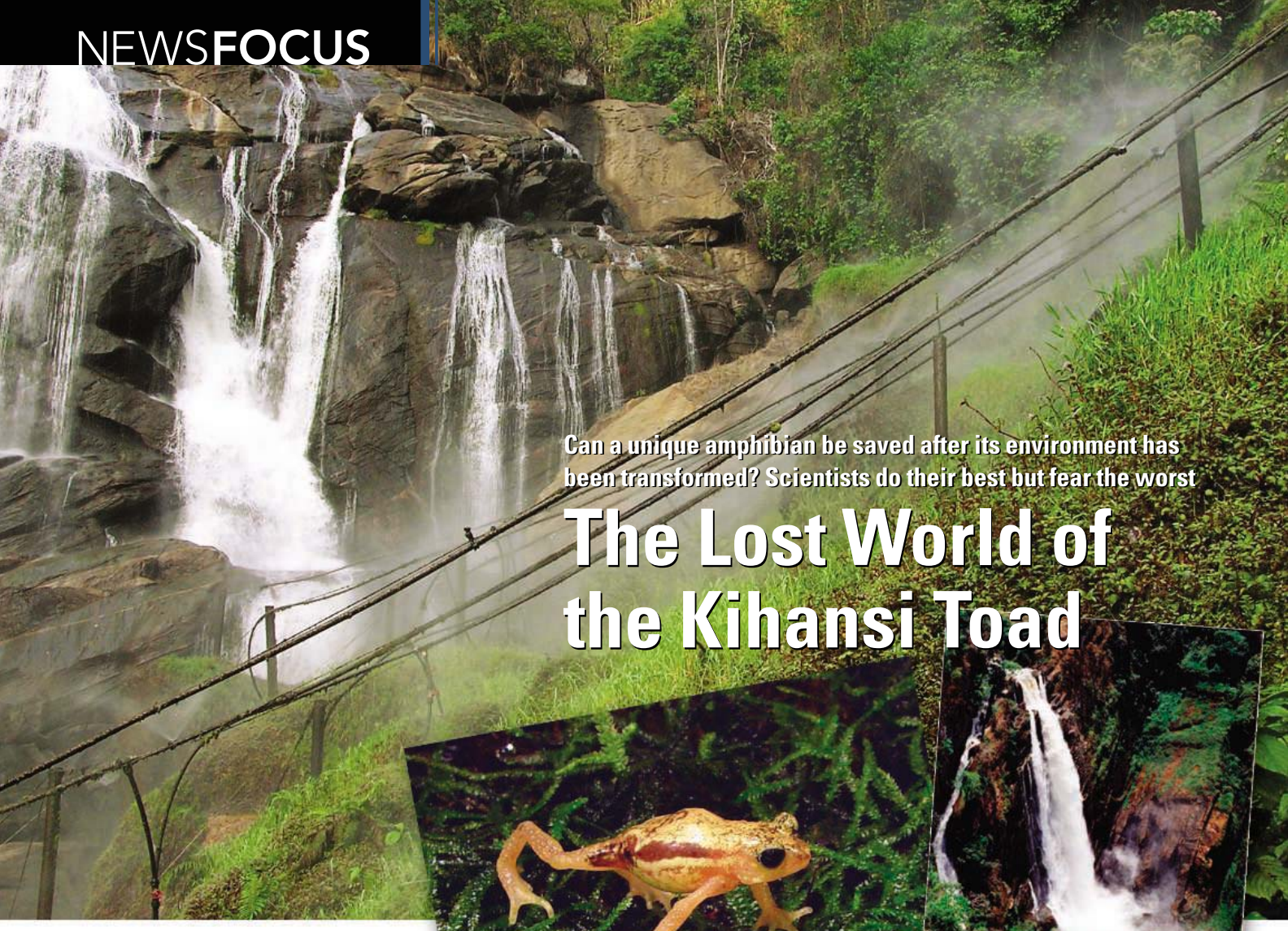
–ROBERT F. SERVICE

Laughlin on the Ropes

SEOUL—Physicist Robert Laughlin, the first non-Korean president of the Korea Advanced Institute of Science and Technology (KAIST), is facing a faculty revolt. Nearly half of the school's 409 professors have voted in an informal tally to unseat him ahead of a meeting of the board of trustees later this month on whether to renew his contract, which comes up for extension in July.

Soon after arriving at the institute in Daejon in July 2004, the blunt-talking Nobelist unsettled some faculty members with a range of reform proposals and funding changes (*Science*, 20 January, p. 321). "Laughlin has done the opposite of what we had asked him to do," says a former dean who stepped down last year after clashing with his boss.

–AHN MI-YOUNG



Can a unique amphibian be saved after its environment has been transformed? Scientists do their best but fear the worst

The Lost World of the Kihansi Toad

BRONX ZOO, NEW YORK CITY—Past the snake exhibit, where gigantic pythons lurk behind thick glass, in the back rooms of the Reptile House, sits a humid, low-ceilinged isolation chamber. Here in five plastic terraria, 159 mustard-colored, fingernail-size amphibians are making what could be their last stand on Earth.

The Kihansi spray toad is 12,800 kilometers from home: Kihansi Gorge, in Tanzania's remote Udzungwa Mountains. For millions of years a great waterfall filled this gorge with perpetual spray and wind, creating a singular environment where the toad and other endemic creatures lived. In 2000, a hydropower dam cut off 90% of the water, and the ecosystem withered. Since then, scores of scientists in many disciplines have performed elaborate, unprecedented deeds to salvage the toad and its lost world. They have managed to raise the toads in captivity, documented the ecosystem's myriad responses to the dam, and engineered in the gorge what may be the world's largest sprinkler system. Their story shows that although human technology can easily upset nature, even the best science may not suffice to restore it.

In splendid isolation

The cool, high peaks of the Udzungwas jut from a sea of dry savanna, forming part of the Eastern Arc Biodiversity Hotspot, a crescent-shaped archipelago of nine mountain ranges. Here are some of the world's oldest rainforests, where long isolation and stable climate have given biota tens of millions of years to evolve. Thousands of plants and animals are endemic to the nine ranges, to one range, or, as in Kihansi, one locale. The spray toad has what may be the smallest range of any vertebrate—2 hectares. Some biologists think it has lived in the gorge or nearby for at least 10 million years.

The gorge begins where the Kihansi River plunges 100 meters off an escarpment, then rushes another vertical 750 meters through 4 kilometers of violent twists and cascades. The river flows year-round, whereas the region's other streams disappear in dry season. The slippery cliffs and the water's ferocity long excluded people, allowing the mist-world creatures to live undisturbed and undiscovered.

Steep drop and dependable flow also are ideal for hydropower. In 1983, engineers envisioned diverting water via a dam above the gorge to a turbine-filled tunnel; flow would bypass the gorge and return to the riverbed at the bottom. A survey of the modest 20-hectare proposed reservoir suggested an environmentally benign project, and in 1994, construction began on the $270 million effort, initially funded by World Bank loans. Development banks in Norway, Sweden, and Germany later joined but insisted that downstream biota be surveyed too.

Thus in 1996, with the dam infrastructure already partly built, biologists including herpetologist Kim Howell of the University of

YYePG Proudly Presents 750+ for Supporth

CREDITS: J. GIBBS/SUNY-ESF; T. HERMAN/BOWLING GREEN STATE UNIVERSITY (TOAD)

Out of water. After a sprinkler system (*left*) replaced the waterfall (*inset*, *right*), Kihansi toads (*inset*, *left*) became vanishingly rare.

Dar es Salaam managed to climb down into several steep, mist-engulfed meadows. Here they found an estimated 50,000 of the skinny, endearing toads, hiding in deep moss mats. Although they have relatives in the region, several unusual features set the toads apart, including flaps over nostrils (possibly to keep out excess spray) and live births (eggs might wash away). Their *chit-chit-chit-chit* call can ramp up to high frequencies inaudible to humans, possibly to overcome constant low-end waterfall roar, says evolutionary biologist Corinne Richards of the University of Michigan, Ann Arbor. The toads ate hundreds of wetland insect species, most still unidentified. Biologists also found at least four new endemic plants in the gorge, including a new coffee species, plus rare trees and threatened primates and birds.

But even as they explored the gorge world, biologists had scant hope for preserving it. "As soon as we found this place, we knew it would be going extinct," says one foreign consultant—who, like several others, feared being quoted by name because of the fierce politics surrounding the dam. To compensate, biologists sought possible toad transplant sites but turned up nothing. They recommended letting half the river's flow continue to the gorge, but that recommendation was not followed. In 1999, European newspapers got wind of unpublished studies, along with the published description of the toad, *Nectophrynoides asperginis*. Groups such as Friends of the Earth accused the banks and Tanzania of violating the International Convention on Biological Diversity, which forbids projects that would wipe out species.

The government and lenders compromised. With an added $6 million loan to cover conservation studies and mitigation, the gorge would get 10% of its previous flow. Part was to be channeled into a several-kilometer-long, gravity-fed pipe system snaking down rock walls to the toad meadows, where hundreds of spray nozzles would spurt mist—a setup meant to mimic natural spray with a fraction of the water. Covering a quarter of the toads' original habitat, the sprinklers are "probably the most highly engineered recovery system for any species ever," says William Newmark, a conservation biologist at the Utah Museum of Natural History advising the World Bank.

But the sprinklers were not ready when the water was to be choked off in early 2000. The shutoff proceeded anyway, and by the time the sprinklers came on 9 months later, the ecosystem had dried up catastrophically. Common plants from adjacent dry areas had invaded former spray meadows; mosses had declined almost 95%; insect diversity had dropped; and only 2000 toads were left alive.

Doing the downstream conservation work only after the dam was well under way was a "huge mistake: Planning was not preceded by a thorough and complete environmental impact assessment," admits conservation biologist Wilfred Sarunday, coordinator of Tanzania's Lower Kihansi Environmental Management Project, which oversees studies and mitigation at the gorge.

In captivity

Fearing the toads would soon be extinct, in December 2000, the Tanzanian government allowed the Wildlife Conservation Society to collect 500 animals for breeding in a half-dozen U.S. zoos. But captive amphibians are difficult to raise, and the animals soon were plagued with lungworms, infections, bone problems, intestinal parasites, and nutritional deficiencies. They would not breed predictably. By spring 2004, the Bronx and Toledo (Ohio) zoos had the only survivors—about 70.

The Bronx Zoo took two unusual steps. It called in the Coriell Institute, a Camden, New Jersey, human genetics outfit that preserves cell lines for research. Their staff created cell lines from dying toads, in hopes that technology would one day permit cloning the cells back into whole creatures. But the cell lines all died. The zoo also farmed out a dozen tiny corpses to Valerie Clark, a Cornell University chemist who studies potentially valuable bioactive substances harbored by amphibians. It was "our last chance" to analyze the toads, says Clark, who plans tests.

Holding on. Kihansi toads now thrive only in zoo terraria (*top*), where keepers managed to get them to breed.

Then, in 2005, the captives perked up. Keepers had devised treatments for various ailments and discovered that although the standard zoo ultraviolet lamps were too big and crude, the toads liked basking in the narrow beams of little 12-volt track-light bulbs. Slowly, the toads started having babies—so small that keepers at first thought they were ants. Now there are about 300 toads between the two zoos.

Meanwhile, in Kihansi, things briefly got better—then much worse. After the sprinklers came on in early 2001, wetland plants slowly regenerated, according to a paper last year in *Biodiversity and Conservation* by Claire Quinn of the University of York, U.K. Some severely affected toad prey such as an endemic *Ortheziola* scale insect also increased, says Peter Hawkes, a consulting entomologist in Pretoria, South Africa. Most encouraging were the toads; internal reports indicate that by June 2003, some 20,000 were hopping about.

A month later, the toads crashed. In August 2003, 40 were seen; in January 2004, only five. Since then, they have virtually disappeared. Once or twice a year, site workers say they hear calls, and in May 2005, a biologist claimed to see one individual. Some scientists say it is still too early to talk about extinction in the wild, but many are pessimistic. "Seeing one spray toad is like … [seeing] one passenger pigeon," says James Gibbs, a herpetologist at the State University of New York at Syracuse who monitors the gorge for the World Bank. "The

CREDITS (TOP TO BOTTOM): T. HERMAN/BOWLING GREEN STATE UNIVERSITY

place is not what it used to be. Nobody wants to say it out loud, but it may be too late."

Biologists point to several possible suspects. The immediate cause may have been chytrid fungus, a deadly skin infection implicated in amphibian crashes around the world, says herpetologist Ché Weldon of North-West University in Potchefstroom, South Africa. His data show that the fungus was absent earlier but present by the crash. One candidate for bringing it in: the imported sprinkler pipes. Another: the boots of dozens of scientists, who traveled in from four continents. Others point out that the 2003 crash coincided neatly with a brief opening of the dam's floodgates to flush sediments. Tests showed these contain pesticides used by a growing number of maize farmers upstream, in concentrations that could kill the toads.

But these are just immediate causes. At bottom, many believe that the gorge environment is broken and can't be reassembled: The changes weakened the toads, and chemicals or infections just finished them off. For instance, the waterfall had constantly replenished spray-meadow soils with wet silt; the sprinklers just sprinkle water, leaving soil crumbly and susceptible to erosion. The waterfall's force also generated ceaseless wind—not supplied by sprinklers—whose now-vanished role in the ecosystem remains unknown. "It's not clear how successful the artificial system is," says water-resources engineer John Gerstle of Hydrosphere Resource Consultants in Boulder, Colorado, who managed much of the environmental work at the gorge until 2004. "It is hard to mimic a situation when you don't necessarily understand it."

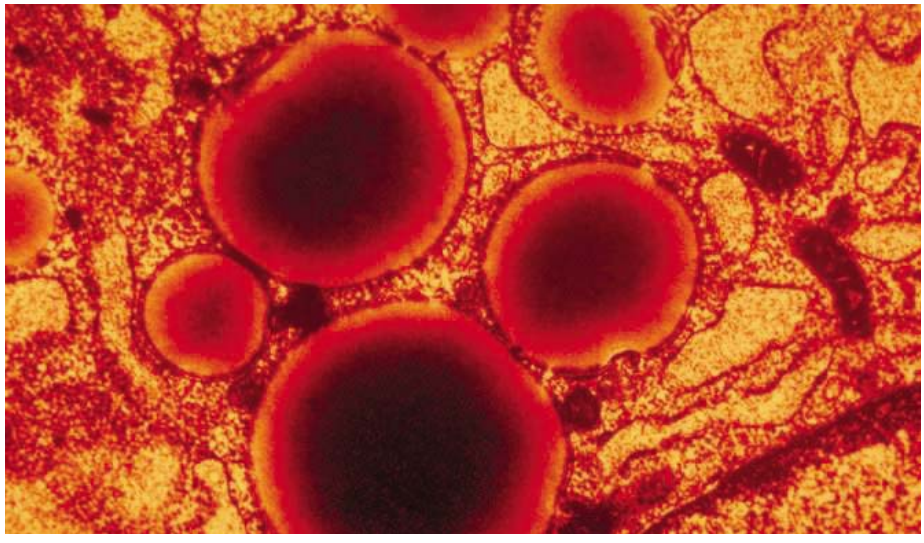
The situation has brought down continuing ire on scientists and their employers. Friends of the Earth President Brent Blackwelder recently wrote to the World Bank: "[Y]our monitoring team is passively documenting the extinction of this unique ecosystem." Sarunday, who still hopes that the system will recover, insists that the banks and Tanzania have "acted in good faith." In one letter to the group, then–World Bank Vice President for Africa Callisto Madavo wrote that measures at the gorge were "designed to ensure an optimal balance between biodiversity conservation and economic development."

The gorge also highlights tensions between developed nations, who funded the dam, and Tanzania, which now gets a third of its electricity from it. Tanzania is one of the most conservation-oriented African nations, but most observers doubt it would have borrowed $6 million for environmental work without pressure from "donor" nations, who want the money repaid. "Most [Tanzanians] say: Who cares about a toad? We want our electricity," says Tanzanian ornithologist Norbert Cordeiro, now at Chicago's Field Museum. When the captive toads were flown on a jet to New York, one Tanzanian newspaper pointed out that few human citizens could expect to do the same. Others question the presence of a seven-person crew doing daily care on the sprinkler system without proof that the toad is there or could ever safely return.

There is perhaps one positive outcome. Tanzania is still rich in biodiversity, and Kihansi has helped develop homegrown expertise to preserve it. The loan has helped Tanzanian and foreign scientists study the gorge together, plus train Tanzanian grad students, hire professors, and buy textbooks and computers. This has "played an important role in capacity-building for local scientists," says Henry Ndangalasi, a botanist at the University of Dar es Salaam. The nation is "mindful of the importance of scientific knowledge," says Sarunday. "The goal of Tanzania is to achieve economic prosperity and have a protected environment at the same time."

–KEVIN KRAJICK

Kevin Krajick is the author of *Barren Lands: An Epic Search for Diamonds in the North American Arctic*.



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CELL BIOLOGY

Great Balls of Fat

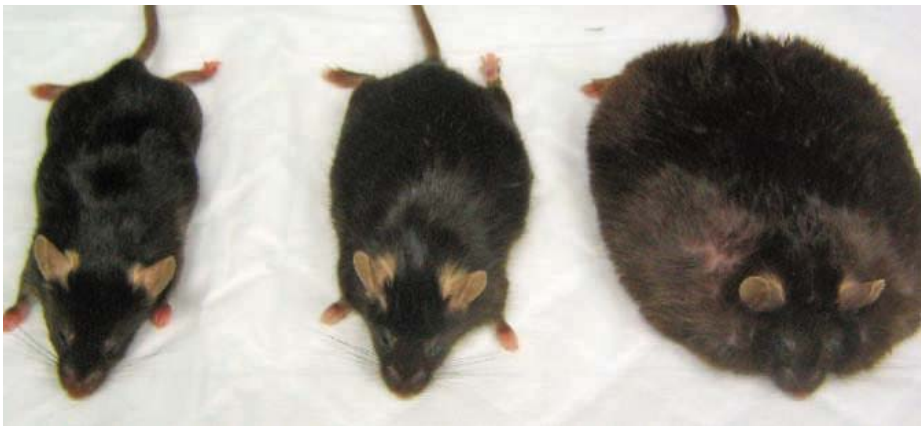
Lipid droplets, long-ignored globules inside cells, are earning recognition as possible organelles involved in cholesterol synthesis and much more

In the breast cells that produce milk, they're called milk fat globules. In plants, they go by the name oil bodies. In fruit flies, lipid storage droplets. Yeast, lipid particles. Cell biologist Richard Anderson prefers the name adiposomes. Immunologist Peter Weller baptized them eicosasomes.

Whatever their name, these intracellular blobs of triglycerides or cholesterol esters, encased in a thin phospholipid membrane, are catching the attention of more and more biologists. It turns out these lively balls of fat have as many potential roles within cells and tissues as they have names. Pockmarked with proteins with wide-ranging biochemical activities, they shuffle components around the cell, store energy in the form of neutral lipids, and possibly maintain the many membranes of the cell. The particles could also be involved in lipid diseases, diabetes, cardiovascular trouble, and liver problems.

This is a far cry from earlier perceptions of lipid droplets, the name most scientists use for the particles. Biologists once considered lipid droplets just inert storage vessels for energy-rich fats. Yet recent studies indicate that the cell keeps a tight rein on their function with molecules that regulate what the particles do, where they go, and what other cellular compartments they cavort with. And a new technique that allows better imaging of lipid droplets in live cells promises even more surprises.

"I've been in cell biology for more than 30 years, and lipid droplets have always been this bag of lipid," says Anderson, who conducts membrane research at the University of Texas Southwestern Medical Center in Dallas. "What is new is the focus on the droplet as an organelle."



Trimming down. Mice that can't respond to the appetite-regulating hormone leptin grow obese (*right*). Mice lacking the perilipin protein that coats lipid droplets burn off the excess fat and become almost as slender (*middle*) as normal mice (*left*).

Knocking out the fat

The first inkling that lipid droplets were more than a cell's beer belly came in the early 1990s. Cell biologist Constantine Londos of the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland, and colleagues identified a novel protein, perilipin, on lipid droplets in fat precursor cells. They also discovered that the cells, when they are stimulated to metabolize the droplet's fat reserves, attach phosphate groups to this protein, suggesting that the cells precisely control the protein's activity during the process.

Whereas perilipin is found almost exclusively in the lipid droplets of fat cells, other researchers soon identified two structurally related proteins—adipose differentiation-related protein (ADRP or adipophilin) and TIP47—associated with lipid droplets in other types of cells. These three became the charter members of the PAT (perilipin/ADRP/TIP47) family of lipid-droplet proteins, whose ranks have since swollen to include more than half a dozen molecules spanning mammals, flies, and amoeba. Researchers in the late 1990s also found a handful of proteins in yeast lipid bodies that are involved in lipid production and degradation.

But what really grabbed everyone's attention were the mutant mice reported in 2000 by Lawrence Chan, an endocrinologist at Baylor College of Medicine in Houston, Texas. Lacking all perilipin thanks to a mutation introduced by Chan's team, these rodents ate more food than normal but burned off two-thirds of the fat a typical mouse would have gained on the same diet. "Their metabolic rate is as if they are exercising all the time," says Chan.

These perilipin knockout mice were a "big breakthrough," says Londos. (His team reported creating its own strain of such mice a few months after Chan's paper was published.) Biochemical experiments by Londos's teams revealed that under normal circumstances, perilipin coats lipid droplets in fat cells and guards their luscious store of lipids. When cells are starved or chemically induced to chew up their fat, an enzyme drapes a phosphate group on perilipin. This changes the protein's shape, exposing the droplet's neutral lipids to degradative enzymes. Finding a way to keep perilipin phosphorylated might prove to be a useful antiobesity therapy, suggests Chan. Londos, however, cautions that "the freewheeling fat breakdown in the perilipin knockout animals" leads to free fatty acids in the blood, a precursor to insulin resistance.

The functions of ADRP and TIP47 on lipid droplets are less well understood. Knocking out ADRP in rodents produced mice that seem to be healthy; cells in the animals compensated by overproducing TIP47, says Londos. His group has since deactivated the genes for both ADRP and TIP47 in mice, but they haven't published the research yet. "You'll have very sick animals if you can't package your lipids," Londos cryptically notes.

Chan is also looking more closely at the ADRP knockout mice to see if researchers missed some subtle problems. Citing yet-to-be-published data, he says that mice lacking ADRP have lower amounts of triglycerides and less fat in their liver cells and are far less likely than typical mice to suffer a fatty liver, a condition commonly found in overweight individuals.

A cholesterol connection?

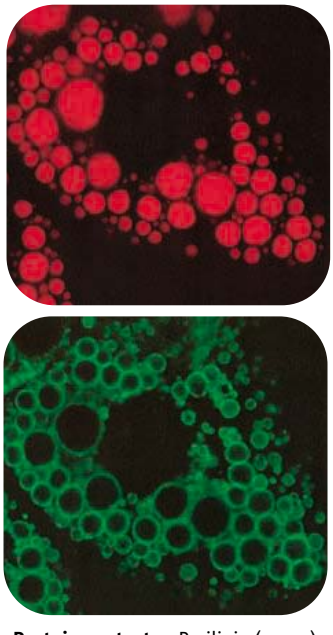
Cholesterol researchers joined the lipid-droplet field in 2001, when three research teams reported that caveolin, a cholesterol-production protein that typically resides in the cell membrane, could be found on the particles under certain conditions.

Just what the protein does there isn't yet clear, however. In one study, cell biologist Robert Parton of the University of Queensland, Australia, engineered cells to make a mutant version of caveolin and found that these proteins amass in lipid droplets, increase the amount of neutral lipids in the cell, and interfere with cholesterol production. This suggested a role for lipid droplets in making cholesterol, instead of just storing its raw materials. But not everybody is convinced because there's no obvious mechanism: Researchers aren't sure exactly how caveolin could help lipid droplets produce cholesterol or get it out of the cell. "I think the clincher finding has yet to be made on the role of caveolin [with lipid droplets] in cholesterol synthesis," says cell biologist Deborah Brown of Stony Brook University in New York.

Caveolin has similarities with other proteins that interact with lipid bodies. "If you squinted hard enough at [its structure], caveolin would look like a PAT family protein," says Brown. But other proteins recently found to hang out with lipid droplets are more diverse. In 2004, several groups surveying the protein profile of lipid droplets revealed that these particles contained dozens of proteins, including ones involved in fat metabolism and in moving membranes between compartments within a cell.

Anderson, who led one of the groups, was so impressed by the droplets' protein ensemble that he argued the particles deserved the name adiposomes to indicate their status as true, metabolically active organelles. Researchers have also found strands of messenger RNA snuggled up to the fatty balls. "Lipid droplets are much more complex than people imagined," says Parton.

The finding that proteins that shuttle membranes around the cell kibitz with lipid droplets startled biologists. Previous reports had placed one such protein, Rab18, in an unrelated cellular compartment. But when cell biologist Toyoshi Fujimoto of Nagoya University Graduate School of Medicine in Japan overproduced Rab18 in liver cells, ADRP disappeared from lipid droplets, and the particles then maneuvered through the cell until they nestled up next to the rough endoplasmic reticulum, the membranous structure upon which ribosomes produce proteins and deposit them into the ER for



Protein protector. Perilipin (green) surrounds bubbles of neutral lipids (red) in fat cells.

CREDITS (TOP TO BOTTOM): PRADIP SAHA AND LAWRENCE CHAN (MICE); TANCY *ET AL.*, *IUMBM LIFE* **56**, 379-385 (2004)

additional processing. Fujimoto says Rab18 could control whether droplets associate with the ER. "We suppose ADRP shields lipid droplets from other organelles so the droplets don't get attached," he says.

Rab18 or one of its cousins may also control fat cells' ability to access the fatty contents of their lipid droplets. Cell biologist Dawn Brasaemle of Rutgers University in New Brunswick, New Jersey, says that when fat cells are eating up their triglycerides for energy, the droplets shatter into smaller fragments, and their protein composition changes massively. "I think a Rab will be a part of that," she says.

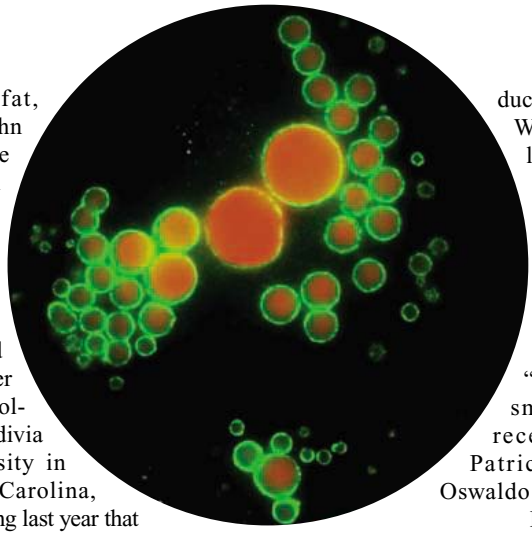
Foaming cells and fatty livers

The medical world has long known that lipid droplets can be problematic: The so-called foam cells that lodge in arteries and contribute to heart disease are not much more than immune cells stuffed with overly large lipid droplets. But recent studies have linked several rare inherited disorders with defects in the function or control of lipid bodies. For example, mutations in the gene encoding a protein called CGI-58 lead to a rare syndrome in which lipids overload a variety of tissues, causing symptoms such as muscle degeneration and scaly skin. Brasaemle and colleagues have found that CGI-58 normally associates with perilipin on lipid droplets but falls off when cells start to metabolize their fat stores.

There are also hints that viruses and bacteria exploit lipid droplets in cells. Livers of mice infected with some varieties of hepatitis C virus bleb with fat, and virologist John McLauchlan of the Medical Research Council Virology unit in Glasgow, U.K., has found that the HCV capsid protein associates with lipid droplets inside liver cells. Also, cell biologist Raphael Valdivia of Duke University in Durham, North Carolina, reported at a meeting last year that when the bacterium chlamydia reproduces inside cells, it coats itself in hijacked lipid droplets.

Peter Weller, who studies lipid droplets at Beth Israel Deaconess Medical Center in Boston, Massachusetts, has also connected the particles to inflammation, an immune reaction that can either damage or protect the body. Weller and his colleagues initially noticed a mere correlation: Immune system cells stimulated to make lipid droplets, by adding free fatty acids to their growth medium, also made eicosanoids such as leukotriene. Inhibit lipid body formation, by withholding such acids, and production of these inflammatory signals dropped.

Then, in 2001, Weller chemically treated immune cells so that any newly synthesized leukotriene would bind to nearby proteins, thus illuminating the eicosanoid's site of production within the cells. When they stimulated lipid body formation, "lo and behold, the leukotriene was on the lipid particle," says Weller, indicating that its production occurred on the droplets' surface. "For us, it was the smoking gun." More recently, Weller and Patricia Bozzo of the Oswaldo Cruz Foundation in Rio de Janeiro, Brazil, have shown the same with other eicosanoids known as prostaglandins.



Disease clue. Mutations in CGI-58 (green), which coats lipid droplets (orange) in fat cells, cause scaly skin and muscle problems.

Additional inflammation mediators fraternize with lipid droplets. Back in 1994, electron microscopist Ann Dvorak of Beth Israel identified cyclooxygenase (COX)—an enzyme that produces prostaglandins—residing on lipid droplets. She has since shown that aspirin interferes with lipid-droplet formation, but in a manner that doesn't depend on the drug's inhibition of COX. "We hope such results might offer some opportunity to find new anti-inflammatory therapies," says Weller.

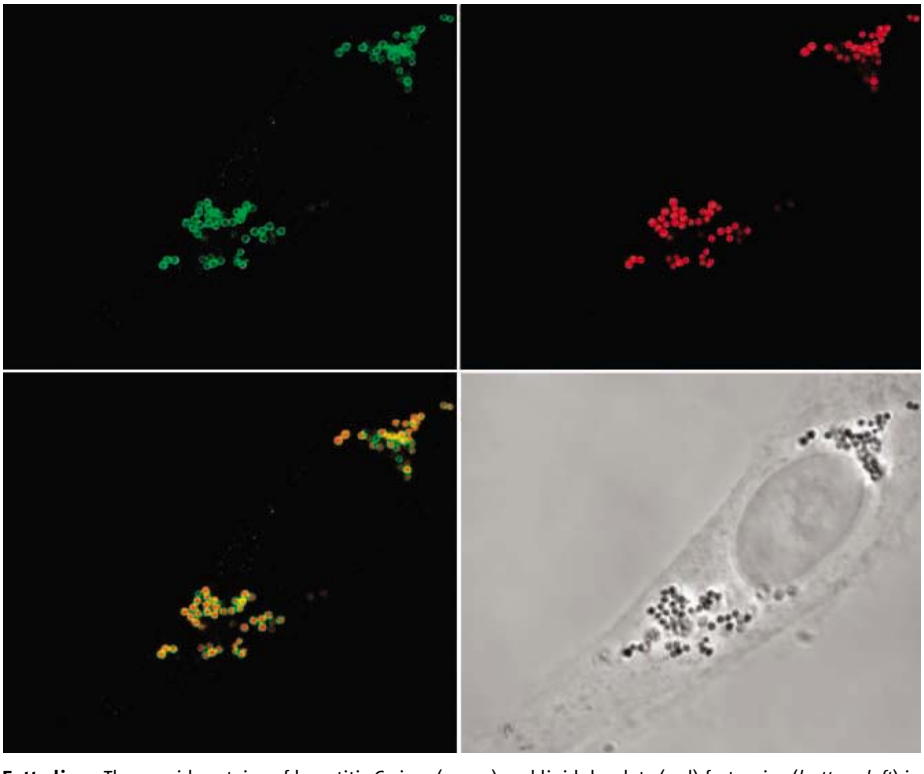
As interest in lipid droplets grows, biologists are searching for better ways to probe the function of the particles. They usually kill cells to isolate and examine the droplets. "One of the things that's hampered their study is there's [been] no technique to study lipid bodies on the long term," says microscopist Emmanuel Beaurepaire.

But in the January 2006 issue of *Nature Methods*, he and his colleagues at École Polytechnique in Palaiseau, France, describe a way to perform real-life imaging of lipid droplets in developing fruit flies and in growing liver cells. While developing light microscopic techniques to visualize mitochondria within cells, they noticed that particular wavelengths lit up lipid droplets surprisingly well.

Whether this new imaging technique will reveal all the secrets of these fatty particles is far from clear. Scientists still don't know if these drops form from other organelles or crop up by themselves. "The biology of lipid droplets is so immense and untapped," says Brasaemle, noting that the first conference will be devoted to them in the summer of 2007. As one of the earliest researchers in the field, Brasaemle is glad that the particles have finally come into their own: She points out that they're discussed in the latest copy of a widely used biochemistry textbook. Ah, but under what name?

–MARY BECKMAN

Mary Beckman is a writer in southeastern Idaho.



Fatty liver. The capsid proteins of hepatitis C virus (green) and lipid droplets (red) fraternize (*bottom left*) in an infected human liver cell (*bottom right*).

CREDITS (TOP TO BOTTOM): SUBRAMANIAN *ET AL., J. BIOL. CHEM.* **279**, 42062-42071 (2004); J. MCLAUCHLAN/INSTITUTE OF VIROLOGY, GLASGOW

WOMEN IN SCIENCE

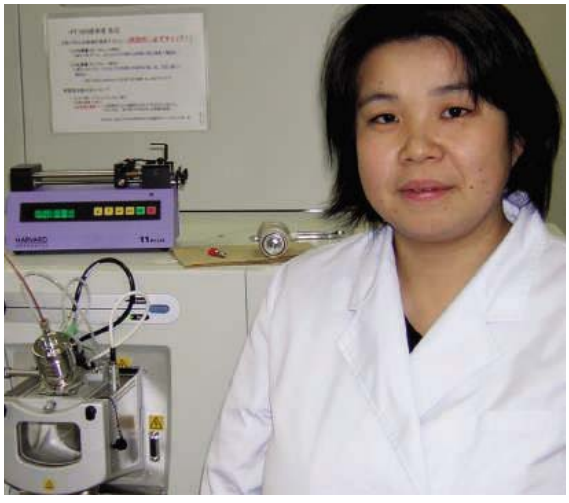
Getting Women Scientists Back on The Career Track in Japan

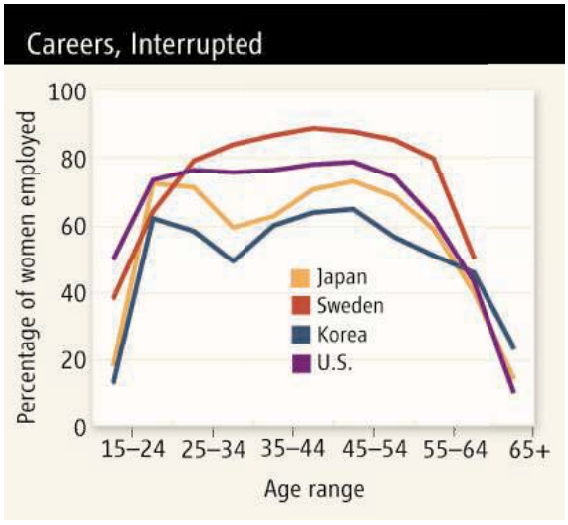
Japan is one of the richest countries, but it's also one where women have little chance of succeeding in science; several new programs aim to end this dubious distinction

TOKYO—Five years after she gave up research to be a full-time mother, Kumiko Usuda is trying to pick up her career where she left off. The Ph.D. astronomer quit working when her first child was born and stayed home until her second child was comfortable with a babysitter. Now living in Hilo, Hawaii, where her husband, also an astronomer, heads engineering for Japan's Subaru Telescope, Usuda does voluntary outreach work for the observatory as she updates her thesis research on molecular gases in galaxies for publication. She would like to arrange observing time at radio telescopes and attend conferences. But it's hard. Although the observatory has given her office space, "there is no financial support for business trips," she says.

Help is on the way. Next month, Japan's government will launch a new category of grants open only to parents returning to the scientific workforce after extended child-rearing breaks. It is part of a package of initiatives that also includes grants for institutions to develop schemes to help women balance research careers and family life. The underlying objective—set out in a draft 5-year policy plan—is to have women claim 25% of all new science and engineering positions at governmental institutions.

Usuda appreciates what the government is doing: "I'm so happy somebody is thinking about my situation," she says. But many worry that the measures will barely dent the formidable barriers that women face. A shortage of daycare facilities and a tradition of long working hours make research careers difficult for mothers with young children. The biggest challenge may be raising the consciousness of senior—primarily male—administrators. Labs and universities "are far from ever thinking of what it takes to be a mother and a scientist," says Kuniko Inoguchi, minister of gender equality and social affairs and a former professor at Sophia University in Tokyo.





Timing is everything. Yoko Iijima had her first child in between postdoc stints. Other women in their 20s temporarily step off the career ladder.

The Japanese government has taken up the gauntlet out of embarrassment, not chivalry. In 2004, women made up only 11.1% of the scientific workforce, the lowest proportion among the 30 member countries of the Organisation for Economic Co-operation and Development. (Portugal has the highest rate, more than 40%; the U.S. figure is 26%.) "This is a very dubious honor for Japan," says Akira Kawamoto, director for science and technology policy in the cabinet office.

The percentage of women scientists has remained low despite rising achievement. In 2004, women made up 23% of those enrolled in science and engineering doctoral programs, up from less than 15% in 1995. Yet few women find permanent academic jobs. At Japan's national universities, the proportion of women holding associate professorships is stuck at about 10%.

To boost the numbers, the Council for Science and Technology Policy (CSTP), the nation's highest science advisory body, set targets for the proportion of newly available permanent positions that go to women in the Third Science and Technology Basic Plan, expected to be adopted by the cabinet this month. Targets vary by field: 30% for health and life sciences, 30% for agricultural sciences, 20% for physical sciences, and 15% for engineering. The percentages are based on the proportions of women currently earning Ph.D.s in each area. "We assume that the ability of men and women are equal, so we can naturally expect that the rates [of women earning Ph.D.s] should be reflected in the rates of women becoming professors or assistant professors," says Kawamoto, who oversaw the drafting of the plan.

The initiative is getting mixed reviews. Plant biochemist Yoko Iijima, a postdoc at the Kazusa DNA Research Institute in Chiba, thinks candidates "should be chosen based on abilities, irrespective of gender." Others argue that gender is an ever-present factor. Mariko Kato, an astronomer at Keio University in Tokyo, says that during initial postdoc fellowships, the male-female ratio closely reflects the proportion of men and women earning Ph.D.s. But as the years pass after graduation, more men find permanent positions, leaving a disproportionate number of women cycling through postdocs or other temporary jobs. Nobuko Wakayama, a protein crystallographer at the National Institute for Materials Science in Tsukuba, says that it is typically the older male scientists who set the tone for institutional decisions on hiring, promotion, and funding. And "they tend to look down on women researchers," she says.

Kawamoto says the targets will make institution heads accountable for helping the nation boost the number of women in responsible research positions. "To change [attitudes], this sort of top-down target is necessary," he says. Although the cabinet office can only rely on "moral pressure," Kawamoto says, it will publicize which institutions are making progress and which aren't.

Beating the "M" curve

To address the day-to-day issues that weigh on women scientists, the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) in the fiscal year beginning in April is launching a trio of new initiatives.

How to balance work and family demands is a challenge for women everywhere. But in

CREDITS (TOP TO BOTTOM): D. NORMILE/*SCIENCE*; SOURCE: INTERNATIONAL LABOUR ORGANIZATION

A $214 Billion Plan of Action

TOKYO—Women researchers are not the only beneficiaries of Japan's Third Science and Technology Basic Plan (see main text). The blueprint for the next 5 years, expected to be finalized and adopted by the cabinet this month, is designed to tackle unfinished business across the R&D spectrum.

The first plan, adopted in 1996, set an ambitious goal of doubling public research spending to ¥17 trillion ($145 billion) over 5 years. That target was achieved. The second plan in 2001 called for ¥24 trillion for R&D, which would raise annual expenditures to about 1% of gross domestic product (GDP). Due to Japan's fiscal woes, however, spending fell short, totaling about ¥21 trillion. For the third plan, policymakers are again eyeing a 1% of GDP benchmark, up from 0.67% in 2003. That translates into ¥25 trillion ($214 billion) for R&D over the next 5 years. Spending increases will depend on economic growth averaging 3.1%, a figure that may be in reach with Japan's economy on the mend.

Highlights of the draft plan include:

Priority areas. The S&T plan aims to continue previous policies of concentrating funding increases in four priority areas: life sciences, information technology, environmental sciences, and nanotechnology and materials sciences. It will also continue to focus on four areas of secondary priority: energy, manufacturing technology, social infrastructure, and frontier sciences (a catchall category).

Fostering competition. To create a more competitive research environment, the plan calls for increasing funding for peer-reviewed grants for individuals and small teams by 30%—up from about $3.3 billion in fiscal 2005—over the next 5 years. And to promote the best institutions, the plan seeks to make universities and labs compete for funds. Currently, they receive most of their funding as block grants based on factors such as enrollment. The goal is for about 30 institutions to break from the pack as world class according to number of citations, says Akira Kawamoto of the cabinet office.

Research fraud. Although details are still being worked out, the final plan is likely to call on universities and institutes to set up offices to investigate allegations of misconduct.

–D.N.

Japan (and Korea), scarce daycare and a cultural bias in favor of mothers staying at home with small children result in an unusual pattern of midcareer dropouts. In most industrial countries, the percentage of women in the workforce stays fairly constant at all ages. In Japan and Korea, however, the percentage peaks for women in their early 20s, dips to a low point for those in their early 30s, and then recovers to the earlier level as they enter their late 30s. This "M" curve of workforce participation plotted against age is deceptive, says CSTP member Reiko Kuroda, a University of Tokyo biochemist. "Women are not able to come back to positions where the quality of the work is the same as before the break," she says. Kuroda suspects that returnees typically end up in jobs with less responsibility and fewer chances to advance to leadership positions.

MEXT officials hope two new programs will get the kink out of the curve. One will challenge institutions to devise novel approaches to balancing motherhood and work. Many women feel pressured to quit a research post rather than take maternity leave because they worry that a prolonged absence will inconvenience colleagues. One possibility is to provide money to hire temps for women on maternity leave. The ministry expects to select proposals from 10 institutions based on a competitive review. Winners will share $12.6 million over 3 years.

The second program will offer research reentry grants to men and women who, like Usuda, put careers on hold to start a family. The $2.2 million program will provide 30 scientists with 2-year fellowships, which are expected to be steppingstones to permanent posts.

Unheralded dilemmas. The new initiatives fail to tackle cultural issues such as late-night lab discussions that mothers must miss, says Miwako Ishido.

CREDITS: D. NORMILE/*SCIENCE*

The third program reaches further up the pipeline. MEXT plans to set up exchanges between high school girls and role-model women scientists and develop brochures on research careers. The ministry has $300,000 for the program in the fiscal 2006 budget. If the programs are successful, it's likely they will be expanded, says MEXT's Masaaki Tanaka.

Women welcome the programs but tend to see them as small steps when leaps are called for. "I really think highly of these initiatives," says cell biologist Miwako Ishido. However, she says, they do little to tackle "the range of cultural issues that make it difficult" for women in research.

Maternity leave isn't likely to improve much, some say. Most women who want to start families are postdocs. "Many women worry that taking maternity leave while on a postdoc won't look good on their resumé," says Iijima. She planned her pregnancy so as to give birth between the completion of a postdoc fellowship at the University of Michigan, Ann Arbor, and the beginning of her current postdoc at Kazusa. "All of my friends are in a dilemma over when to have kids," she says. Ishido adds that many institutions have an age limit—typically 35—for candidates for permanent positions, adding a twist to the child-rearing puzzle.

One of the biggest headaches remains unaddressed: "We need childcare centers in labs and universities," says Inoguchi. Only two of Japan's dozen or so top research universities have on-campus daycare. Tohoku University opened the first last fall, and Nagoya University will follow suit in April. The Nagoya nursery will keep children until 9 p.m. But that may not be late enough for what Ishido calls "the night-owl culture" of Japan's labs. Researchers typically arrive late in the morning and work until midnight. "All the most interesting lab discussions take place late at night," she says.

The slow pace of change in the academic community has already pushed many talented women in other directions. Ishido, who earned her Ph.D. at Kyoto University and did a postdoc stint at the Scripps Research Institute in San Diego, California, chose to get off the postdoc treadmill when she returned to Japan last year. She now splits her time between benchwork at a biotech start-up and evaluating high-tech investment opportunities for a venture-capital firm. "This trend of talented women pursuing opportunities outside academia is likely to push universities to change," she says.

But the extra nudge from the new clutch of programs is welcome. Usuda says she regularly checks the Web site of the Japan Society for the Promotion of Science, where details of the grants for women returning after career breaks are due to be posted. The new programs may not usher in an era of equality, but they send a strong message that the status quo is no longer acceptable.

–DENNIS NORMILE

The new world. Ioannis Miaoulis says students need to understand engineering as well as science to succeed.

CREDIT: BOSTON MUSEUM OF SCIENCE

PROFILE: IOANNIS MIAOULIS

A Passion for Teaching Leads to Engineering Change in Schools

Most U.S. students aren't exposed to engineering until college. Massachusetts is different—and Ioannis Miaoulis is a big reason why

Eighteen years ago, Ioannis Miaoulis took a wrong turn on his way to Tufts University and ended up in the parking lot of a middle school outside Boston. Instead of asking for directions to the Medford campus, where he was an assistant professor of mechanical engineering, Miaoulis walked into the principal's office and offered to demonstrate the principles of superconductivity, a hot field that he was exploring. One week later, Miaoulis was showing eighth grade students how a magnet could float in the air above a superconductor.

That classroom session launched the Greek-born researcher on a parallel career in science education that has made him a passionate advocate for technological literacy. Disturbed by a curriculum that contained "so much about flowers and rocks and nothing about planes and power plants," Miaoulis started a statewide campaign to introduce engineering concepts into schools. In 2001, Massachusetts education officials made their state the first to include engineering in its curricular standards and student assessments. "Miaoulis was the one who made that happen, no question about it," says Massachusetts education commissioner David Driscoll. "He sold engineering to us in a way that demystified it and made a compelling case for teaching it to kids from an early age."

Today, Miaoulis, 44, has expanded that campaign into a national effort. In 2003, he left academic life to become president of the Museum of Science in Boston. It houses his National Center for Technological Literacy (NCTL), a nonprofit organization with $32 million from businesses and the federal government that has developed an elementary school curriculum and an engineering course for high school students. Last fall, schools in a dozen states began trying out the elementary school curriculum, and high schools in seven states are piloting the advanced course. "My dream is to have the humanmade world be a part of the curriculum of every school in the country within the next 9 years," says Miaoulis. "I say nine because last year I said 10."

A prized speaker at education summits around the country, Miaoulis promotes the cause of precollege engineering education like nobody else. His monomaniacal focus can even be a little annoying to others in the field. "He thinks he discovered the idea of teaching engineering to kids," says Kendall Starkweather, executive director of the International Technology Education Association (ITEA) in Reston, Virginia. ITEA was founded in 1939, and in 2000, it issued national standards for technological literacy. But Starkweather doffs his hat at Miaoulis's achievements. "The bottom line is that he has succeeded in getting one state to adopt engineering standards and helped to focus national attention on the E and T in STEM."

A passion for teaching

Miaoulis's own acquaintance with engineering started at home: His father was a civil engineer. After moving to the United States as a teenager, Miaoulis got both his bachelor's and doctoral degrees in engineering from Tufts and eventually joined its faculty. But he was disturbed by the public's ignorance about what engineers do, as well as its higher regard for scientists. "People who drive trains and repair VCRs are considered engineers," he says. Even the old building that houses the National Academy of Engineering "has a janitor's closet that says 'Engineering' on it," he notes.

At Tufts, Miaoulis's passion for teaching made him immensely popular and raised engineering's profile on campus. For example, to teach heat transfer, he became a cooking instructor, providing students with lamb recipes alongside energy-rate equations. His tasty lessons reduced the traditionally high attrition rates for first-year engineering students to the point at which the department began to attract majors from the liberal arts.

But Miaoulis wasn't content to confine his teaching talents to a college campus. Working with local public schools in the late 1980s made him realize that "98% of the curriculum is focused on the natural world, even though 98% of the things that most people interact with in their daily lives (apart from their own bodies) are humanmade." It seemed crazy to him that students "spend days learning how a volcano works but no time learning how a car works." Then he delivers the punch line: "How often do they find themselves in a volcano?"

When Miaoulis was appointed to a panel revising the state's science and technology standards in 1998, he saw the opportunity to do something about his pet peeves, but he knew he needed allies. So he reached out to the state's association of technology education teachers, many of whom had been losing jobs as schools closed down printing and automotive shops to fund computer labs. "Going to science teachers did not seem like a good idea because teachers that are well-fed and secure—why would they change anything?" he says. "I thought, if I partner with tech-ed teachers and make the

Brushing up on technology. Second graders at Barbieri Elementary School in Framingham, Massachusetts, learn about the technology behind everyday items such as a toothbrush.

case that adding engineering could upgrade their whole profession and save their jobs, I'd have the backing of that entire community."

It didn't go that smoothly. Many tech-ed teachers without engineering degrees worried that they'd be left behind. Others thought that science teachers would be asked to carry the load because of the strong math and science foundation needed. Indeed, state officials did try to throw technology out of the standards, arguing that shop skills such as metalworking and woodworking did not belong in higher level academic standards. Miaoulis convinced them that tech ed would become as academically relevant as physics when blended with engineering. "He showed a lot of political savvy during the process," says Driscoll. "He made a connection with people—from the governor to state education officials—and he was relentless in a nice way."

Technical difficulties

Since his successful advocacy for precollege engineering in Massachusetts 5 years ago, Miaoulis has delivered talks in more than 25 states and lobbied hundreds of politicians and school administrators. But no state has yet followed Massachusetts's lead. Even within the state, most middle and high schools have been hard-pressed to implement the new standards. One hurdle is the lack of clear guidelines in the standards and the absence of curricular materials for the middle school grades, which NCTL is currently developing. But a tight budget and a finite school year also pose serious problems, says James Surowski, head of the science department at Forest Park Middle School in Springfield, Massachusetts.

"In the 185 days available during our school year, an eighth grade science teacher already has to cover the Earth's history, change in ecosystems over time, the Earth and the solar system; … the list goes on," says Surowski. "Now the same teacher—we can't hire a specialty engineering teacher—must make time for technology topics as well. The pond just got a lot wider and a lot shallower." Laura Bottomley, who leads the American Society for Engineering Education's K–12 project, notes that "we have trouble getting schools to teach science, let alone engineering."

> **"He sold engineering** … in a way that demystified it and made a compelling case for teaching it to kids."
>
> —David Driscoll

Some Massachusetts teachers worry that many high school students may not have a sufficient foundation in mathematics and physics to benefit from the engineering course. "We're currently designing a model deck for which students need to calculate live loads and dead loads, which requires algebra," says Richard Skrocki of Shepherd Hill Regional High School, who is implementing the high school engineering course developed by NCTL. "I can see that some of my students who are weak in math are having difficulty. But I don't have the time to teach them algebra before proceeding with the class."

At the same time, other educators say that NCTL's engineering course for high schools is not rigorous enough. "When they teach students music, they don't give them cardboard models of musical instruments. Why should engineering be any different?" asks Richard Blais, vice president of Project Lead the Way (PLTW) in Clifton Park, New York, which offers a demanding middle school and a precollege engineering program.

Miaoulis, who calls PLTW's course the "Cadillac" program, admits that the NCTL curriculum, including the use of cardboard models in the deck-building assignment, is more basic than what many engineers would like to see. But he thinks it gives them a good sense of engineering design and problem solving. "Once this thing catches on," he says, "we can create more specialized courses that involve calculus and advanced physics."

In the meantime, Miaoulis tries to help teachers fit engineering into their existing curricula. After taking a 2-week summer course offered jointly by Tufts, the Museum of Science, and Worcester Polytechnic Institute, Debbie Warms asked her seventh graders at Charlton Middle School to create assistive devices for students with disabilities. "They had to use math skills such as measurement, ratios, and proportions, and solve equations with a variable and geometry," says Warms, who found the exercise time-consuming but worthwhile.

That blending of disciplines is exactly what engineering can bring to precollege education, says Miaoulis: "It can bring to life not just the math and science but also the social studies, the English skills." As an example, he points to NCTL's pilot elementary school curriculum that combines history, geography, and culture into an engineering lesson. In one book, Yi Min learns about the use of materials engineering in building and preserving the Great Wall in her native China. Another lesson follows Aisha, a young Boston girl, as she explores a local potato chip factory with her father and learns about industrial engineering.

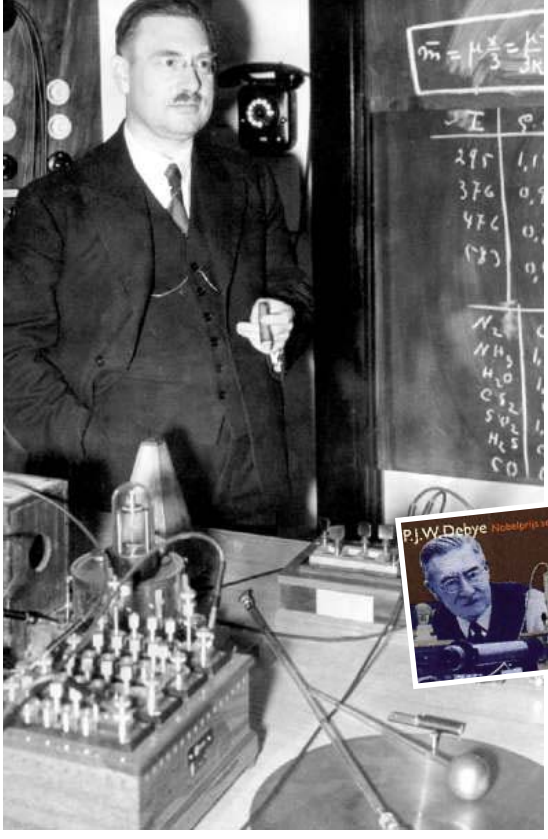
Miaoulis believes that approach will boost undergraduate engineering enrollment and increase diversity by making the subject more relevant to students' lives. And he's unapologetic about the program's potential impact on other subjects now being taught. "Okay, you might have to cut other things from the curriculum a little bit," he says. "But then, so be it. Look at what you add."

–YUDHIJIT BHATTACHARJEE

Engineering study receives NSF support

CREDIT: Y. BHATTACHARJEE/*SCIENCE*

EDITED BY YUDHIJIT BHATTACHARJEE



On Campus

FALLEN FROM GRACE. Two universities in the Netherlands have distanced themselves from Dutch physicist and 1936 Nobel laureate Peter Debye after new revelations about Debye's closeness to the German Nazi regime. Utrecht University said last week that it will rename its Debye Institute—a decision the institute director calls "hasty"—and Maastricht University will no longer award the Peter Debye Prize for science unless the foundation sponsoring the award renames it.

Debye succeeded Albert Einstein as director of the Kaiser Wilhelm Institute for Physics in Berlin in 1934 and remained until 1939, when he left and took a job at Cornell University. Although Debye was known to have helped expel Jews from the German Physical Society, which he chaired in 1938, he has often been painted as an apolitical figure. But in a recent dissertation, science journalist and historian Sybe Rispens claims Debye displayed considerable loyalty to the Nazi regime, signing personal letters with "Heil Hitler" and offering to return to Berlin as late as 1941. Rispens also discovered a letter showing that Einstein tried to prevent Debye from getting a U.S. job.

Maastricht University has announced a new, more thorough study of Debye's life; the former Debye Institute will undertake one as well, says its director, Leo Jenneskens. He says he would have preferred to keep the name for now, but he was overruled by the university board. And the American Chemical Society, which has an annual Peter Debye Award for Physical Chemistry, is looking into the matter as well.

MOVERS

SUDDEN EXIT. Last year, when a budget crisis at Brookhaven National Laboratory threatened the existence of its Relativistic Heavy Ion Collider (RHIC), lab director Praveen Chaudhari met with U.S. politicians and "anyone who would listen" to argue for more money for the lab. But now that the proposed 2007 federal budget restores funding for RHIC, Chaudhari, 68, has decided not to stick around to enjoy his success. He'll be leaving 30 April, after 3 years on the job, to return to research at the Department of Energy (DOE) lab on a part-time basis.

"It's probably best for the institution since I've drawn so much flak for my protests over RHIC," says Chaudhari, who accepted a private donation of $13 million to run the facility this year. Other researchers complained that the gift set a bad precedent for public funding of science, he says.

Robert Jaffe, a physicist at the Massachusetts Institute of Technology in Cambridge, says Chaudhari's single-minded advocacy for the lab likely ruffled the feathers of some higher-ups. "He's built a very firm foundation for physics there," Jaffe says, "and he probably broke some eggs to make that omelet."

NAVAL RESEARCH HEAD. The Navy's $1.8 billion Office of Naval Research has a new commander in Rear Admiral William Landay, who took over from Jay Cohen in January.

Trained as a systems engineer, Landay's previous job was head of the Navy's applied research wing in shallow water and mine warfare. But despite his background in developing and acquiring new technology, Landay told *Science* through a spokesperson that he's "not so acquisition-oriented [that he'll] undermine basic research." Among the challenges he wants to focus on are basic and applied research on improvised explosive devices and the "anthropology of terrorism."

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CREDITS (TOP TO BOTTOM): AP PHOTO; PITT POST; JOHN WILLIAMS/OFFICE OF NAVAL RESEARCH; BNL; UCSD-TV

<< Two Cultures

LARGER THAN LIFE. In college, Ivan Schuller decided that physics was easier than acting, his original major. Decades later, the University of California, San Diego (UCSD), professor has found a forum for his inner ham in a television show about nanoscience.

When Things Get Small is a half-hour program about the physicist's real-life quest to develop the world's smallest magnet. Created through a collaboration between Schuller (above, left), UCSD-TV producer Rick Wargo, and actor Adam Smith (above, right), the show uses humorous gimmicks to explain concepts from the nanoworld. In one scene at a baseball stadium, Smith buys a bag of peanuts from John Moores, owner of the San Diego Padres, before explaining that the number of atoms in a single human hair equals the number of peanuts needed to fill all 30 major league baseball stadiums.

Schuller also illustrates the minute nanoscale by yanking hairs from the actor-host's head and going nose-to-trunk with a shrunken elephant. "We want people to go away and think that science is fun, entertaining, and maybe a little bit useful," he says. The show will premiere this month on the University of California's public satellite broadcast service.

LETTERS

edited by Etta Kavanagh

Crucial Choices for the Nascent ERC

THE DIVERSE SCIENTIFIC COMMUNITIES SUPPORTING THE INITIATIVE FOR SCIENCE IN EUROPE (ISE) welcome the steps taken toward establishing the European Research Council (ERC), notably, the appointment of a Scientific Council of 22 outstanding scientists. Many important decisions must be taken in the coming months to ensure that the ERC meets the high expectations of the community as a truly autonomous agency that funds fundamental research in all disciplines on the basis of scientific excellence, while guaranteeing that the public funding provided for it will be prudently managed.

The choice of legal structure for the ERC will be vital. An "Executive Agency," established and staffed predominantly by employees of the European Commission (EC) recruited through open competition and detachment, is one option; the alternative is a structure that is independent of the EC but in which all member states are represented. The ISE agrees with the pragmatic choice of an Executive Agency structure, at least for the start-up phase of the ERC, with the possibility of changing the legal structure following an independent assessment after 3 to 5 years.

"Many important decisions must be taken in the coming months to ensure that the ERC meets the high expectations of the community as a truly autonomous agency that funds fundamental research in all disciplines on the basis of scientific excellence, while guaranteeing that the public funding provided for it will be prudently managed."

Despite the Commission's role in establishing the agency, the ERC must be substantially independent of the EC and, crucially, must be allowed to function outside the standard procedures of the Framework Programmes. In this regard, the leading role of the new Scientific Council must be rigorously respected; the Executive Agency must act under the authority of the Scientific Council. As a consequence, it appears imperative to us that the choice of the director of the Executive Agency must be based on proposals made by the Scientific Council. The alternative, whereby the EC chooses the key officers, would put at risk the trust between the Scientific Council and the Executive Agency that will be essential to earn, in turn, the trust and respect of the wider scientific community.

The new ERC has the opportunity to engage European researchers in a way that the Framework Programmes have so far failed to do. The Executive Agency must grasp this opportunity by choosing procedures that best serve the needs of science in Europe: Applications must be evaluated solely on scientific merit, the application and reporting procedures must not overburden scientists with administration, and funding must be through grants, like those of the national funding agencies, rather than, as is currently the case in the Framework Programme, through contracts with rigid deliverables and milestones, which are counterproductive to the unpredictable frontier research.

Finally, although no decision on the level of financing of the next Framework Programme has been announced, we know that budget negotiations point to a significant reduction in funds for research by the European Union, possibly including the ERC. In any event, the ERC must have a budget that is commensurate with the important task it is to fulfill: to support basic research and increase the competitiveness of Europe. This budget should be at least €1 billion per year in the first years and grow quickly to €1.5 to 2.0 billion per year (the size of the larger national research council budgets) within the 7-year Framework Programme. A smaller budget than this could seriously undermine the ERC. Funding of this magnitude, i.e., at least €9 billion, should be earmarked for the ERC in the Framework Programme budget.

The temptation to reduce ERC funding to protect existing actions, however valuable, or to transfer to the ERC the charge of delivering other parts of the Framework Programme (without the associated budget) must be resisted. If the budget is inadequate, the success rate of applications will be too low, many important projects will not be funded, and the best researchers will not apply for grants or participate in the peer review process. All of these would doom the nascent ERC.

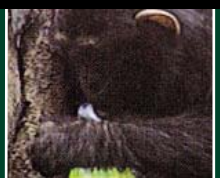
THIS LETTER IS ENDORSED IN A PERSONAL CAPACITY BY THE PRESIDENTS, CHAIRS, AND DIRECTORS GENERAL OF 57 EUROPEAN ORGANIZATIONS IN ALL SCIENTIFIC DISCIPLINES UNDER THE AEGIS OF THE INITIATIVE FOR SCIENCE IN EUROPE (FOR FURTHER INFORMATION, SEE WWW.INITIATIVE-SCIENCE-EUROPE.ORG). THE COMPLETE LIST OF SIGNATORIES IS AVAILABLE AT WWW.SCIENCEMAG.ORG/CGI/CONTENT/FULL/311/5765/1240B/DC1.

Objectivity in Science

OBJECTIVITY IS A CORNERSTONE OF SCIENCE. Bias can erode objectivity when unwittingly introduced into the reporting and teaching of discoveries and theories. This is evident in articles and books on evolution today and may contribute to difficulties in the acceptance of evolution by many supporters of intelligent design.

Science has not yet developed to the point of being able to assign purpose to activities in the natural world. In fact, it may never develop to that level. Yet purpose is often implied in descriptions of DNA replication, and this introduces bias.

Scientists generally agree that there is no purpose in evolution. The evolutionary process moves along as a result of interactions among and between components of various levels of organization: populations, organisms, molecules, atoms, and subatomic particles and



A helping hand
1248

Early Mesoamerican writing
1249

waves. If purpose does exist, its discovery is outside the realm of science at this time.

Describing the production of a mutation, such as a DNA strand with a base sequence not complementary to the template strand at each base point, as an "error" or "mistake" unwittingly ascribes purpose to the process. It introduces the assumption that a new strand is "supposed" to be complementary to the template strand at each base point. Such a biased assumption is outside the realm of science. One could just as easily assume that a new complementary strand is not supposed to be an exact complement, but rather a source of variation. This assumption is also outside the realm of science.

Base-pairing during replication occurs as a result of natural attractions and repulsions between partially charged components of the bases. This is true if the new strand becomes an inexact complement just as much as it is true if the new strand becomes an exact complement. An inexact complement should not be considered a "mistake."

This may appear trivial at first glance, because scientists often communicate among themselves informally, using purposeful language while not intending a literal interpretation (e.g., elements try to achieve an outer octet of electrons). The danger lies, however, in the use of such informal language in articles and books intended for nonscientists, including textbooks used in high schools and colleges. When mutations are not presented as natural phenomena, but rather as "mistakes," it becomes difficult for a nonscientist to view them objectively.

Many supporters of intelligent design find discomfort in the concept that humans have evolved as a result of "mistakes." Although it is not an obligation of scientists to address discomfort in concepts, it is an obligation of scientists to present findings in an objective, scientific manner. Presenting mutations as "mistakes" should not be avoided due to any discomfort that may occur. Presenting mutations as "mistakes" should be avoided simply because such a presentation does unwittingly introduce purpose, and hence bias, to the concept. People being presented with the case for evolution should be allowed to evaluate objective arguments, without having first to overcome what they may consider a negative bias, when that bias should not have been introduced in the first place.

KENNETH R. GORDON

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Influenza Mutation from Equine to Canine

IN THEIR REPORT "TRANSMISSION OF EQUINE influenza virus to dogs" (21 Oct. 2005, p. 482), P. C. Crawford *et al.* observed an unprecedented interspecies transfer of a complete equine influenza virus to the dog and the emergence of a new canine-specific influenza virus associated with acute respiratory disease. They noticed that a viral hemagglutinin (HA), a critical determinant of host species specificity of influenza virus, differs mainly in four residues (N83S, W222L, I328T, and N483T) between the equine and canine HA orthologs, out of which only one (W222L) is exposed to the serum and is most likely involved in receptor binding. Our analysis revealed an additional important mutation (N54K) located in the antibody-binding region of HA (*1*). This residue is highly conserved in all noncanine (94) HA sequences of the subtype H3N8 (see multiple sequence alignment at http://mvg.bioinfo.pl/supplemental). In contrast, a leucine residue observed in the canine HA at position 222 is also present in three equine orthologs deposited in GenBank. The figure presents a comparison of the three-dimensional models of the equine and the canine HAs created with 1HA0 (*2*) and 1KEN (*3*) structures as templates. Highlighted areas show that the N54K mutation changes the electrostatic potential on the protein surface significantly. Moreover, it is placed in the middle of an N glycosylation motif (Asn-X-Ser) and likely increases the probability of the posttranslational modification of the preceding asparagine (*4*). The glycosylation of HA has been shown to enable the virus to mask its antigenic sites (*5*). We suggest that this mutation may help the virus escape the dog's immune defense and may be part of the minimal repertoire of changes required for the host specificity transition in the observed case.

MARCIN VON GROTTHUSS AND LESZEK RYCHLEWSKI

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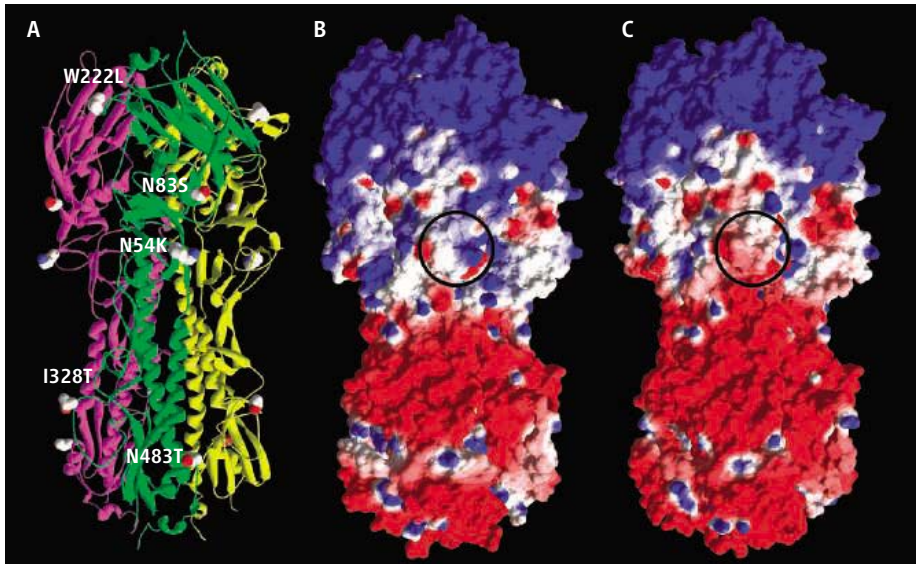
References and Notes

1. D. Fleury, R. S. Daniels, J. J. Skehel, M. Knossow, T. Bizebard, *Proteins* **40**, 572 (2000).
2. J. Chen *et al.*, *Cell* **95**, 409 (1998).
3. C. Barbey-Martin *et al.*, *Virology* **294**, 70 (2002).
4. S. H. Shakin-Eshleman, S. L. Spitalnik, L. Kasturi, *J. Biol. Chem.* **271**, 6363 (1996).
5. J. J. Skehel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1779 (1984).
6. M.v.G. is a fellow of Foundation for Polish Science.

Response

GLYCAN CAMOUFLAGING OF HA ANTIGENIC sites is certainly a successful strategy of the human influenza virus in evasion of antibody responses elicited by previous influenza infections in adult populations. Canine influenza is a newly emerging pathogen and dogs are immunologically naive to the virus. Without the selective pressure applied by preexisting antibodies, the role of the amino acid substitution at position 54 in virus escape from antibody neutralization is probably not as important in either adaptation to or maintenance of the virus in the canine population at this time.



The ribbon representation (**A**) and the protein surface colored by electrostatic potential (**B**, **C**) of 3D models of the canine (A, B) and the equine (C) influenza hemagglutinins. Five dog-specific mutations are marked (A) with visible amino acid side chains. Highlighted areas (B, C) show the highest differences in electrostatic potential caused by the N54K mutation. This picture was created with Swiss PDB Viewer.

We agree with von Grotthuss and Rychlewski that, in addition to the four amino acid substitutions we described, the N54K substitution in the HA may have contributed to the successful transfer of equine H3N8 virus to the dog. However, the effects of these amino acid mutations on HA function are undefined and are likely multifactorial. It will be very interesting to monitor the evolution of these five sites of the HA as the virus becomes endemic in the dog population and herd immunity develops from infection or vaccination.

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CORRECTIONS AND CLARIFICATIONS

News Focus: "New neurons strive to fit in" by G. Miller (17 Feb., p. 938). Two names are misspelled in the photo credit on page 938. The correct credits are Verónica Piatti, Nicolás Morgenstern, and Alejandro F. Schinder. In the diagram on page 939, the labels "GABA input" and "Glutamate input" are reversed. GABA should be yellow, and glutamate should be blue.

Reports: "A clonogenic bone marrow progenitor specific for macrophages and dendritic cells" by D. K. Fogg *et al.* (6 Jan., p. 83). The affiliations were incorrectly numbered. The complete correct author list and affiliation list follow: Darin K. Fogg,[1] Claire Sibon,[1] Chaouki Miled,[1] Steffen Jung,[2] Pierre Aucouturier,[3] Dan R. Littman,[4] Ana Cumano,[5,6] Frederic Geissmann[1,7]

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Policy Forum: "Social values and the governance of science" by G. Gaskell *et al.* (23 Dec. 2005, p. 1908). The table referred to in the following sentence was not the one on p. 1909, and it is now included in the SOM as table S2: "The distribution of people in the United States, Canada, and Europe who opted for each principle of governance is shown in the table (p. 1909)."

Research Articles: "Animal evolution and the molecular signature of radiations compressed in time" by A. Rokas *et al.* (23 Dec. 2005, p. 1933). The list of supporting online material did not appear at the end of the reference list. It should read as follows:
Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5756/1933/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 to S8
References

This Week in Science: "Turning slightly faster" (26 Aug. 2005, p. 1297). The last sentence of this item is incorrect. It should read: "A systematic offset in seismic waves that pass through the inner core demonstrates that it is indeed rotating faster than the rest of the planet by about 0.3 degrees to 0.5 degrees per year."

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

MICROBIOLOGY

Of Microbes, Medicine, and Ecology

James Strick

The early antibiotics were universally heralded as magic bullets and miracle drugs. But in 1942 René Dubos, one of the discoverers of these new compounds, understood enough about ecology and natural selection to warn that microbes would inevitably develop resistance and, furthermore, that resistance would be hastened if the drugs were overused. Enveloped in the optimism of the early molecular age, the medical community, including most researchers, did not listen to the warning. In many ways, this episode captures the essence of Dubos's scientific career: always the harbinger of a complex, "both good news and bad news," ecological understanding of humans' relationship to our pathogens and the rest of our environment; usually a decade or two ahead of the world in seeing the big picture.

René Dubos, Friend of the Good Earth
Microbiologist, Medical Scientist, Environmentalist

by Carol L. Moberg

American Society for Microbiology, Washington, DC, 2005. 292 pp. $29.95. ISBN 1-55581-340-2.

Interestingly for a scientist of Dubos's stature, *Friend of the Good Earth* is the first published, in-depth biography. The book's subtitle shows where the red thread of his work led him, from agronomic engineering to microbiology to medical research to environmentalism. Dubos (1901–1982) was initially inspired by the writings of the Russian environmental microbiologist Sergei Vinogradskii. He came to the United States in 1924 to study under Jacob Lipman and Selman Waksman. After working with Oswald Avery at the Rockefeller Institute, he went on to lead his own team of researchers there.

As Dubos's personal secretary for much of the last phase of his career, Carol Moberg is in a unique position to know about her subject. She has also consulted such archival sources as exist. But she points out that Dubos, a very private person, up until the early 1970s regularly purged and disposed of all his correspondence—an exasperating situation for a biographer who wishes to do a fair job. Fortunately, the record for Dubos's last decade is more complete.

Dubos was a polymath, but as Moberg ably shows, his various agendas are not as disconnected as might first appear. From his earliest encounters with microbes, Dubos tended to view them—whether in the soil or interacting with a host in a medical context—from an ecological point of view, much as Louis Pasteur had. It was natural that he would eventually take an interest in Pasteur's work, and his interest produced an empathetic yet critical biography (*1*). Thinking ecologically led to Dubos's very early prediction of antibiotic resistance. It also led him to recognize the importance of subclinical infections, to see limits on medicine's ability to "eliminate human pathogens," and (like E. O. Jordan and Theobald Smith before him) to take an interest in bacterial variation and the earlier, more extreme doctrine of pleomorphism (the supposed ability of bacteria to change shape dramatically). In a 1952 book (*2*), Dubos and his wife Jean commented that tuberculosis "is not likely to be solved by the use of any drug." A 1959 book (*3*) warned of the resurgence of old, "conquered" diseases; he predicted the emerging epidemics of today and, to some extent, "Darwinian medicine."

CREDIT: LAWRENCE R. MOBERG

Trying to understand how to create a philosophy of prevention, Dubos's thinking led him to Lao Tzu and other philosophers. He realized that it was far easier to change smoking habits, the design of industrial equipment, and the planning of cities (i.e., social pathologies that medical advice was unlikely to reach) than to spend much greater amounts of energy on medical intervention after the fact—trying to clean up the mess of lung cancer, industrial accidents, and infectious diseases spread by poor sanitation. He warned early about "biological Freudianism"—e.g., that low birth weight from poor maternal diet or exposure to pesticides in utero could biologically impair animals for life. By the 1960s, Dubos's broadening understanding of such issues made him a leader in the emerging environmental movement.

Moberg's Dubos is a highly sympathetic figure, both as a scientist and as a humanist. It seems clear that Dubos was very foresighted and thoughtful. Yet on at least one occasion, Moberg's sympathy for the man interferes with a more complex portrait (such as a professional historian would strive to construct) of Dubos's role in U.S. biological warfare research during World War II. Although all of what Moberg reports is true, she appears to be trying to suggest that Dubos's involvement was "only as a consultant," "on background research," and in defensive work (especially on vaccines). Whereas Dubos may have only been a consultant on some matters, historian Gerard Fitzgerald has shown that he was the head of "Project Y" (the work on dysentery). As Moberg reports, *Shigella* dysentery was not in the end weaponized, but that was only because it did not work, not for lack of trying. Fitzgerald has shown that there was no firewall of any kind between defensive and offensive work and that a very large number of academic microbiologists participated in the program (*4*). This historical work is not accusatory in tone. Rather, its purpose is to make us aware that scientists have a long history of working for governments during wars and that such situations have always been fraught with ethical ambiguities—a lesson that has no less relevance in our own time. No one is served by whitewashing this ambiguous part of a scientist's career, even if it is done with the best intentions. Indeed, this episode needs to be restored, because (as with many other prominent microbiologists involved in the work) "one finds no trace of Dubos's wartime bioweapons research among his publications or in biographical information published after his death" (*5*).

Moberg's only serious shortcoming, then, is having overlooked a number of relevant recent history of science dissertations [not least Jill Cooper's scientific biography of Dubos (*6*)] that could have substantially enriched her story. Otherwise, *Friend of the Good Earth* is a fine book. It offers a well-crafted introduction to the life and career of a scientist whose work has never been more relevant than now—in a world with HIV, SARS, amphibian chytridosis, and avian flu.

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References

1. R. Dubos, *Louis Pasteur, Free Lance of Science* (Little, Brown, Boston, 1950).
2. R. Dubos, J. Dubos, *The White Plague: Tuberculosis, Man, and Society* (Little, Brown, Boston, 1952).
3. R. Dubos, *Mirage of Health: Utopias, Progress, and Biological Change* (Harper, New York, 1959).
4. G. J. Fitzgerald, thesis, Carnegie Mellon University (2003).
5. G. J. Fitzgerald, *Recent Sci. Newsl.* **2**, 1 (Fall 2000).
6. J. E. Cooper, thesis, Rutgers University (1998).

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ASTROBIOLOGY

Deities for Atheists

Michael Shermer

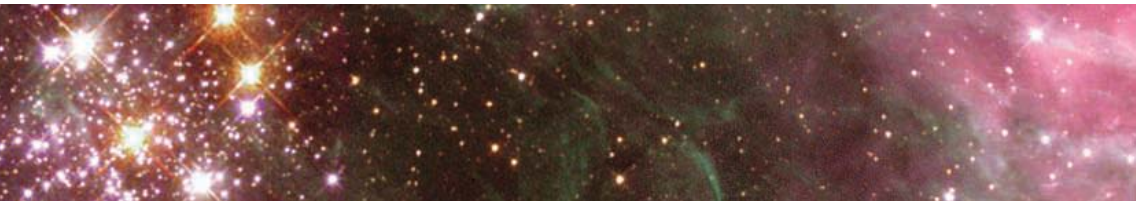
On 8 February 2000, the *New York Times* science section highlighted a new book (*1*) by paleontologist Peter Ward and astronomer Donald Brownlee, who were called radicals for challenging the orthodox assumption that the cosmos is probably teaming with complex life. "Now, two prominent scientists say the conventional wisdom is wrong" (*2*).

How did the search for extraterrestrial intelligence (SETI) change from the heresy it was in the early 1960s (when Frank Drake, Carl Sagan, and others took it up) to "conventional wisdom" by the late 1990s? It certainly was not due to any new empirical data, as SETI continues to be a science without a subject. A compelling answer may be found in George Basalla's critically important

Civilized Life in the Universe
Scientists on Intelligent Extraterrestrials

by George Basalla

Oxford University Press, Oxford, 2005. 247 pp. $29.95, £17.95. ISBN 0-19-517181-0.



Civilized Life in the Universe, the best treatment on the history and science of the subject since Steven Dick's magisterial two volumes (*3*, *4*).

Basalla's tightly woven and highly readable narrative begins with an epigraph from the theoretical physicist Paul Davies: "What I am more concerned with is the extent to which the modern search for aliens is, at rock-bottom, part of an ancient religious quest." That is precisely what it is, says Basalla, a historian of science and technology at the University of Delaware. He proceeds to outline three assumptions that underlie thinking about extraterrestrial intelligence from antiquity to the present: the universe is very large or infinite, there are other inhabited worlds, and these other complex and intelligent beings are vastly superior to us.

Modern cosmology has confirmed the first assumption. We live in an accelerating expanding universe some 13.7 billion years old, which contains several hundred billion galaxies, each of which houses several hundred billion stars. And modern astronomy is in the process of confirming half of the second assumption: there are a great many worlds circling those hundreds of billions of stars in our galaxy. Whether they are inhabited or not, of course, remains to be seen.

As for the third assumption, if we did make contact with an ETI, they would have to be vastly superior to us (since we just recently mastered radio and spaceflight). On an evolutionary time scale, an ETI species only slightly ahead of us biologically could be millions of years ahead of us technologically. Pace Arthur C. Clarke, I have called this Shermer's last law: "Any sufficiently advanced extra-terrestrial intelligence is indistinguishable from God" (*5*).

Basalla notes that this is actually an ancient belief: "The idea of the superiority of celestial beings is neither new nor scientific. It is a widespread and old belief in religious thought.… Aristotle divided his universe into two distinct regions, the superior celestial realm and the inferior terrestrial realm." The incorporation of Aristotle into Christian theology carried this belief into the Middle Ages. "Christians populated the celestial regions with God, the saints, angelic beings of varying ranks, and the souls of the dead. These immortal celestial beings were superior to mortals, who inhabited the inferior terrestrial realm." Even though the Copernican revolution overturned Aristotelian cosmology, "the belief that creatures living on a distant planet were superior to the human species" hung on into the modern age, and "religious elements continue to adhere to the perception of extraterrestrial life even as we study it in the twenty-first century."

My analysis of SETI pioneers found that most were once religious but became either atheists or agnostics as adults (*6*). Radio astronomer Frank Drake—creator of the canonical "Drake Equation" for estimating the number of ETIs inhabiting the galaxy—was raised Baptist and later reflected: "A strong influence on me, and I think on a lot of SETI people, was the extensive exposure to fundamentalist religion" (*7*). Drake has suggested that "immortality may be quite common among extraterrestrials" (*8*). Carl Sagan—who did more than anyone to conventionalize SETI—was raised Jewish and became agnostic. He wrote of SETI's importance, "It touches deeply into myth, folklore, religion, mythology; and every human culture in some way or another has wondered about that type of question" (*7*). ETIs are secular gods. Deities for atheists.

Why should so many people—theists and atheists, theologians and scientists—believe in the existence of superior celestial beings, be they angels or aliens? Basalla's answer is twofold: First, as the psychologist Robert Plank suggests (*9*), humans have an emotional need to believe in imaginary beings: "Despite all their scientific trappings, the extraterrestrials discussed by scientists are as imaginary as the spirits and gods of religion or myth." Second, as Steven Dick has proposed, when the Newtonian mechanical universe displaced the spiritual world of the Middle Ages it left a vast and lifeless void, which modern science then filled with ETIs. Basalla considers Sagan's vision of alien intelligences: "Sagan was certain that these creatures were benevolent. They would help us solve current problems, like the spread of nuclear weapons and environmental pollution, by sharing their advanced knowledge with us."

The author is also highly critical of the anthropomorphism inherent in SETI. Although Sagan identified a number of chauvinisms (oxygen, carbon, temperature, etc.) that cloud scientific thinking on the subject, Basalla thinks that he didn't go far enough. The chauvinism that ETIs will communicate via radio signals, that their intelligence will take a form similar to ours, and, especially, that they are social beings who live in civilizations are anthropomorphic assumptions without any scientific foundation. Given that we cannot even communicate with terrestrial intelligences such as apes and dolphins, Basalla wonders "how can we hope to decode complex messages sent by superior extraterrestrial ones?"

Nevertheless, if we do make contact with intelligent celestial beings, all of this speculation and conjecture will fall by the wayside in favor of real science. So in the spirit of scientific inquiry, the search must go on. Ad astra!

References

1. P. D. Ward, D. Brownlee, *Rare Earth: Why Complex Life Is Uncommon in the Universe* (Copernicus, New York, 2000).
2. W. J. Broad, *New York Times*, 8 February 2000, p. F1.
3. S. J. Dick, *Plurality of Worlds: The Origins of the Extraterrestrial Life Debate from Democritus to Kant* (Cambridge Univ. Press, Cambridge, 1982).
4. S. J. Dick, *The Biological Universe: The Twentieth-Century Extraterrestrial Life Debate and the Limits of Science* (Cambridge Univ. Press, Cambridge, 1996).
5. M. Shermer, *Sci. Am.* **286**, 33 (January 2002).
6. M. Shermer, *The Borderlands of Science: Where Sense Meets Nonsense* (Oxford Univ. Press, Oxford, 2001).
7. D. Swift, *SETI Pioneers: Scientists Talk About Their Search for Extraterrestrial Intelligence* (Univ. Arizona Press, Tucson, 1990).
8. F. Drake, D. Sobel, *Is Anyone Out There?* (Delacorte, New York, 1992).
9. R. Plank, *The Emotional Significance of Imaginary Beings: A Study of the Interaction Between Psychopathology, Literature, and Reality in the Modern World* (Thomas, Springfield, IL, 1968).

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PUBLIC HEALTH

A National Tuberculosis Archive

Translation of tuberculosis research into benefits for citizens, clinical practice, and policy formation would be facilitated by development of an integrated resource.

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Currently, no disease has the type of large-scale, systematic biological and informatic integration that permits researchers to cross easily between field-relevant and research-relevant isolates in the context of clinical, epidemiological, and phylogenetic characterizations. This is due, in large part, to the intense demands systematic data collection and organization place on clinicians and the public health apparatus. However, the complete population-based data collection infrastructure necessary for such a resource is already in place for tuberculosis (TB) in the United States.

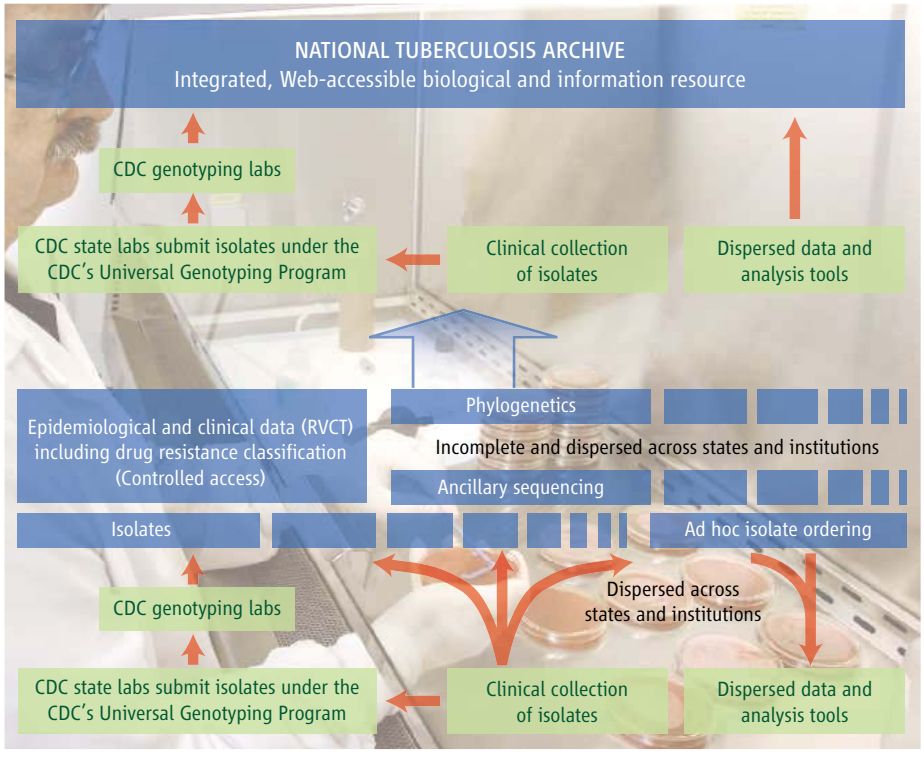
Enhanced online at www.sciencemag.org/cgi/content/full/311/5765/1245

About one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB) (*1*). TB disproportionately burdens the world's poorest countries (*2*, *3*). The threat of emerging multidrug-resistant (MDR) strains (*4*) is severe. The number of TB cases in the United States is relatively small: just under 15,000 per year (*5*). Yet TB is fundamentally a "transnational" disease, with more than half of all U.S. cases occurring in non–U.S.-born persons (*5*). Schwartzman *et al*. (*6*) estimate that under current practices the United States will spend about $2 billion over the next 20 years just treating immigrants from Mexico. And although "only" 15,000 cases is a public health success story compared with historic epidemics, indolence in efforts to combat the disease would be unwise (*7*). It is estimated that cutbacks in TB-related resources in the late 1970s and 1980s contributed to a resurgence in TB among predominately immunocompromised and socially marginalized patients that cost more than $1 billion to control in New York City alone (*8*).

Every verified TB case in the United States is reported to the Centers for Disease Control and Prevention (CDC), along with clinical and epidemiological information, in a document called the Report of Verified Case of Tuberculosis (RVCT) (*9*). In 2004, CDC began a program to genotype a MTB isolate from every patient reported in the United States under its TB Universal Genotyping Program (*10*, *11*). Other laboratories already have substantial information on strains from countries in which epidemiologic trends are well described (*12*) or drug-resistant MTB is epidemic (*13*, *14*). The genome of MTB has been sequenced (*15*). Collections of genotypic, epidemiological, and/or clinical data are available in electronic databases but are not integrated, and phylogenetic data relating strains are incomplete. What is missing is an integrated, comprehensive, population-based biologic and informatic resource that can drive evidence-based decision-making.

We propose creation of a National Tuberculosis Archive, a comprehensive repository of characterized *M. tuberculosis* isolates along with their genomic, clinical, and epidemiological data (see figure, this page). Such an integrated resource would close the loop between clinical isolates and research data, allowing users to search on metadata criteria and to obtain samples of isolates matching field-relevant criteria. Molecular variation could be readily linked with phenotypic characteristics, and geographic distribution with temporal sampling. Bench scientists could explore fundamental questions about the relation between molecular variation and clinical consequences, health-care providers could alter patient care on the basis of strain-specific pathogen properties, and public health officials could track outbreaks across jurisdictions and back through time. Disparate data would be integrated in a Web-accessible platform for easy access.

Archiving etiologic material along with an integrated information resource has previously proved to be a prescient step in public health preparedness, as was seen in the 1993 hantavirus epidemic when museum archives of rodent sera and tissue samples were crucial in demonstrating that the virus had been widely endemic for years (*16*–*18*). This gave public health policy-makers invaluable baseline information to determine appropriate and targeted responses, while removing biowarfare concerns.



Differences between the current configuration of clinical isolates, research strains, and data and the proposed National Tuberculosis Archive (Bottom). Current unintegrated configuration.

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Results from prior molecular epidemiologically based efforts are a harbinger of the value of a comprehensive national archive for TB. A population biologic analysis of 10 years of data in San Francisco suggests that strains of *M. tuberculosis* may spread more efficiently in human populations when they are within the sympatric populations in which they evolved (*19*). So knowing an outbreak's characteristic molecular and phylogenetic signature can help in identifying new human ethnic groups at risk. A clinical study in New York City suggests that patients afflicted with specific clades of bacteria manifest a more profound disease (*20*, *21*). Other public health jurisdictions are seeing the full extent of unsuspected transmission and the need for new interventions (*22*). For the MDR-TB outbreaks caused by strain W in New York in the early 1990s, availability of archived samples linked to public health surveillance data enabled investigators to identify the origin of strain W, trace its acquisition of drug resistances, track its spread in New York City and around the country, and develop public health control measures (*8*, *23*, *24*).

The RVCT-based public health infrastructure and CDC Universal Tuberculosis Genotyping Program are already in place. We estimate the cost of integration for TB to be $15 million over 3 years.

Because *M. tuberculosis* is a human pathogen, but a poor candidate for bioterrorism, it is an excellent pilot for a more systematic program of human pathogen socioecological-genomic characterization. Improvements in disaster preparedness will result from a more focused and thoughtful integration of science, medicine, and public health.

References

1. World Health Organization (WHO)/South East Asia Region Facts (w3.whosea.org/tb/faqs.htm).
2. "Global tuberculosis control: Surveillance, planning, financing" (WHO, Geneva, 2005); (www.who.int/tb/publications/global_report/2005/en/).
3. "Addressing poverty in TB control" (WHO, Geneva, 2005); (www.who.int/tb/publications/2005/en/).
4. M. Abdel Aziz *et al*., "Anti-tuberculosis drug resistance in the world: third global report 1999–2002" (WHO, Geneva, 2004); (www.who.int/tb/publications/2005/en/).
5. U.S. Department of Health and Human Services (HHS), Centers for Disease Control and Prevention (CDC), *Reported Tuberculosis in the United States, 2003* (CDC, Atlanta, GA, September 2004).
6. K. Schwartzman *et al*., *N. Engl. J. Med*. **353**,1008 (2005).
7. J. D. H. Porter, K. P. W. J. McAdam, *Annu. Rev. Public Health* **15**, 303 (1994).
8. T. R. Frieden, P. I. Fujiwara, R. M. Washko, M. A. Hamburg, *N. Engl. J. Med*. **333**, 229 (1995).
9. CDC, "Reported tuberculosis in the United States" (CDC, Atlanta, GA, 2004; 2005); or see Technical Notes, Reported Tuberculosis in the United States, 2004; (www.cdc.gov/nchstp/tb/surv/surv2004/default.htm).
10. HHS, CDC, *MMWR Morb. Mortal. Wkly. Rep.* **54**(02), (2005).
11. National TB Controllers Association/CDC Advisory Group on Tuberculosis Genotyping, "Guide to the application of genotyping to tuberculosis prevention and control" (CDC, Atlanta, GA, June 2004); (www.cdc.gov/nchstp/tb/genotyping/toc.htm).
12. K. DeRiemer *et al*., *Lancet*. **365**, 1239 (2005) [with Editorial].
13. S. Shin *et al*., *Microb. Drug Resist.* **11**, 26 (2005).
14. L. L. Han *et al*., *Int. J. Tuberc. Lung Dis.* **9**, 818 (2005).
15. S. T. Cole, *Nature* **393**, 537 (1998).
16. J. M. Hughes, C. J. Peters, M. L. Cohen, B. W. J. Mahy, *Science* **262**, 850 (1993).
17. J. S. Duchin *et al*., *N. Engl. J. Med*. **330**, 949 (1994).
18. T. L. Yates *et al*., *Bioscience* **52**, 989 (2002).
19. S. Gagneux *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 2869 (2006).
20. S. S. Munsiff *et al*., *Emerg. Infect. Dis.* **8**, 1230 (2002).
21. S. S. Munsiff *et al*., *J. Infect. Dis*. **188**, 356 (2003).
22. M. Kato-Maeda, P. M. Small, *West. J. Med*. **172**, 256 (2000).
23. P. J. Bifani *et al*. *JAMA* **275**, 452 (1996).
24. T. B. Agerton *et al*., *Clin. Infect. Dis*. **29**, 85 (1999).

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MEDICINE

A Portfolio Model of Drug Development for Tuberculosis

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Because of inadequate funding and the lack of promising drugs, no new antituberculosis drugs are likely to become available before 2010.

More than 10 million people develop tuberculosis (TB) annually, and about 2 million die each year (*1*, *2*). Forty years have passed since the last novel anti-TB drug, rifampicin, was introduced. Treatment requires difficult, multidrug regimens for a minimum of 6 months. Rates of multidrug-resistant cases are increasing, particularly in settings where directly observed therapy and standardized drug regimens are not used consistently and where supplies of anti-TB drugs are frequently interrupted (*3*, *4*). New drugs that offer improvements over current therapies are desperately needed.

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Public-private partnerships are promising efforts to combat the global burden of infectious diseases (*5*). Public sector and philanthropic organizations support research and management of drug portfolios while accessing the infrastructure and expertise of the pharmaceutical industry. The Medicines for Malaria Venture was the first such partnership (*6*), and the model has been successful in the campaign against river blindness in West Africa.

In 2000, the Global Alliance for TB Drug Development (TB Alliance) was established to spearhead development of new anti-TB therapies. The TB Alliance establishes partnerships between industry, governments, and academia and manages a portfolio of compounds in various stages of discovery and testing. The TB Alliance has publicly stated a goal of bringing a novel anti-TB drug to market by 2010 (*7*, *8*). According to the strategic plan of the Stop TB Partnership, the current global TB drug pipeline consists of 27 compounds. The TB Alliance manages two of the compounds in clinical testing and numerous others in discovery (*8*).

What is the likelihood of bringing a new TB drug to market by 2010? Pharmaceutical firms commonly evaluate drug development efforts using a "portfolio model," a structured process based on principles of decision analysis (*9*–*11*). The approach allows companies to value their research-and-development efforts and make resource allocation decisions. We developed a Monte Carlo simulation model to evaluate drug development from the perspective of a public-private partnership (*12*). Our model permits calculation of the expected number of successful compounds, expected costs at each stage of development, and all expected development costs for successful and unsuccessful compounds.

Inputs to the model include success probabilities, clinical trial costs, and durations for each stage of drug development (*12*). In calculating expected costs of clinical trials for a given compound, we assumed that the development process follows the standard framework of preclinical through phase III testing. The model also includes the rate of return used to discount future cash flows. We also examined the expected costs for clinical development in Uganda compared with the United States.

First, we used the global TB drug portfolio for clinical trials performed in the United States, which includes four compounds in preclinical development, five compounds in phase I, and two compounds in phase II (*8*). The likelihood that the portfolio will generate at least one successful compound is ~73% by year 14 (2019)

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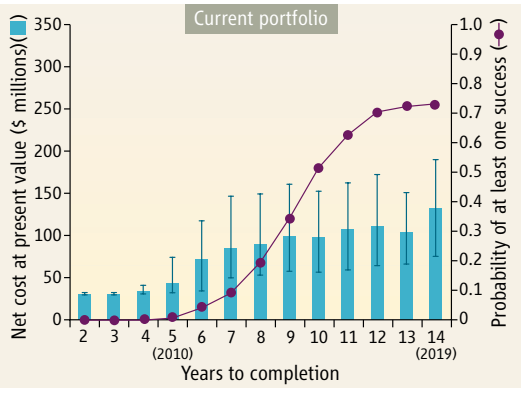


Simulation model for the likely global TB drug portfolio in 2005.

(see figure, this page, top). However, the likelihood of the portfolio's generating at least one successful compound by 2010 is less than 5%.

As expected, when we doubled the number of phase I and II compounds in the portfolio, the cumulative success probability for generating at least one compound was higher (93% versus 73%). However, the probability of developing a novel anti-TB compound by 2010 remained less than 5%.

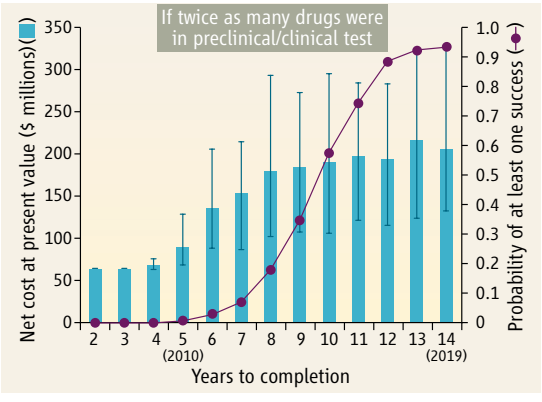
Given that there is a probability of failure at each stage of clinical testing, coupled with the long lag time between discovery and conclusion of phase III testing, the TB Alliance and its partners, to meet their stated goal, must either quickly acquire several compounds (i.e., more than 10) in phases II and III of clinical development or achieve the capacity to oversee parallel phase II trials of different compounds in the hope of organizing a few large phase III trials. The likelihood that such a large number of advanced compounds would become available is infinitesimally small. It is more likely that the number of compounds would increase through discovery and the introduction of new compounds into early clinical testing. Even in this case, a large number of new candidates (i.e., more than 20) would be necessary to ensure a high likelihood of generating a new drug (see figure, this page, bottom). It would likely be nearly 10 years before any of these compounds reached the market.

The model also provides information about the costs likely to be incurred in bringing new anti-TB drugs to market. For example, suppose that the estimated global TB drug portfolio generates a successful compound in year 10. The mean net present value of the development costs for this drug is estimated at $98 million, with a range of $56 million to $152 million. This figure does not include discovery costs, which have been estimated to account for one-quarter of total drug development costs (*13*). Moving clinical trials to countries with emerging economies, such as Uganda, could reduce this cost to $45 million.

Our model further suggests that, in the absence of any compounds currently in phase II trials, the TB Alliance would need 30 compounds in phase I testing to be 95% confident of generating at least one successful drug. Clinical testing in this scenario could take 12 years and cost as much as $400 million. The TB Alliance's estimates, from which many of our cost inputs were derived, predict that the costs of developing a new drug for TB range from $120 million to $240 million. Given that the TB Alliance has an estimated $36 million in cumulative funding through 2007 (*14*, *15*), our analysis implies an estimated shortfall likely to exceed $100 million for the first compound (*12*) and even more if we wanted to be confident of developing a single new therapy.

The findings underscore the need for strengthening collaborative research and development between the public and private sectors. Indeed, the TB Alliance recently announced a partnership with GlaxoSmithKline to develop four novel classes of potential anti-TB agents. The TB Alliance has also established formal relations with several academic medical centers and pharmaceutical firms, the Novartis Institute for Tropical Diseases, and experienced clinical trials groups, including the TB Trials Consortium of the Centers for Disease Control and Prevention. Our findings speak to the potential value of adopting a "product development" approach in the public sector when the goal is to address within a short period of time a specific public health need through the development of new clinical therapies.

Increased funding and cost-sharing strategies are needed as well. At the 2006 meeting of the World Economic Forum, the Bill & Melinda Gates Foundation announced a tripling of its commitment to TB eradication efforts to $900 million over the next decade. Continued support of this kind can help narrow the funding gap for TB drug development. However, drug development will require substantial investments from the National Institutes of Health (NIH) and agencies in other countries. Current NIH funding for TB is about 1/20th the funding for HIV/AIDS and 1/10th the funding for biodefense. We estimate that a drug portfolio designed to produce a single successful compound would require a commitment of up to $400 million. This estimate includes only clinical development costs, and not the costs of distributing a new drug and educating health workers about its use.



Simulation model if the number of compounds in preclinical and clinical testing in 2005 is doubled.

Conducting trials in developing and transitional countries (where most TB cases are found) may be an attractive option for reducing costs. However, there are obstacles that a public-private partnership must consider. The most significant is the lack of infrastructure needed to conduct trials using best practices. Thus, for phase III trials, laboratories have to be built or revamped, with appropriate safeguards; equipment has to be ordered; taxes (formal and informal) have to be paid for importation; and personnel have to be trained to conduct the tests and quality-assurance activities. Although investments in clinical research capacity in these settings are critical for future drug development, and costs will diminish over time, private industry has not traditionally been willing to develop such sites. Public support for fixed infrastructure will be essential to the development of new therapies. Although the current drug pipeline and levels of funding are unlikely to yield a novel drug by 2010, the TB Alliance's success at bringing public and private parties together and nurturing the drug development process makes the future more promising than the past.

References and Notes

1. E. L. Corbett *et al.*, *Arch. Intern. Med.* **163**, 1009 (2003).
2. World Health Organization (WHO), Tuberculosis, fact sheet no. 104 (WHO, Geneva, 2005); (www.who.int/mediacentre/factsheets/fs104/en/).
3. B. J. Seaworth, *Infect. Dis. Clin. North Am.* **16**, 73 (2002).
4. A. Zumla, *Lancet Infect. Dis.* **1**, 199 (2001).
5. Global IDEA Scientific Advisory Committee, *CMAJ* **171**, 1203 (2004).
6. R. G. Ridley, *Lancet Infect. Dis.* **2**, 54 (2002).
7. Global Alliance for TB Drug Development (www.tballiance.org/).
8. Working Group on New TB Drugs, Stop TB Partnership, *Strategic Plan: Prepared for the Global Plan to Stop TB: 2006-2015*; (www.stoptb.org/wg/new_drugs/documents.asp).
9. J. S. Stonebraker, *Interfaces* **32** (6), 77 (2002).
10. R. F. Tiggemann, D. A. Dworaczyk, H. Sabel, *Drug. Inf. J.* **32**, 813 (1998).
11. P. Sharpe, T. Keelin, *Harv. Bus. Rev.* **76** (2), 45 (1998).
12. See supporting material on *Science* Online for methods.
13. Pharmaceutical Research and Manufacturers of America (PhRMA), *Pharmaceutical Industry Profile 2001* (PhRMA, Washington, DC, 2001).
14. *Research and Development for Neglected Diseases: Lessons Learned and Remaining Challenges* (International Federation of Pharmaceutical Manufacturers Associations, Geneva, 2004).
15. R. Widdus, K. White, *Combating Diseases Associated with Poverty: Financing Strategies for Product Development and the Potential Role of Public-Private Partnerships*. (Initiative on Public-Private Partnerships for Health, London, 2004).

16. We thank D. Seils for editorial assistance and V. DePuy for assistance with the success probabilities model.

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Supporting Online Material
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BEHAVIOR

Who Are More Helpful, Humans or Chimpanzees?

Humans, including infants, are more willing than closely-related chimpanzees to cooperate and behave altruistically and cooperatively, probably in part accounting for their evolutionary success.

Joan B. Silk

Do you hold the door for shoppers laden with packages? If you received two copies of the latest issue of *Science* in the mail, would you give the extra one to a colleague or throw it in the recycling bin? Do you make donations to charity, serve on departmental committees, recycle bottles, or donate blood? If you are like most people, you help in these sorts of situations and are motivated by empathy and concern for the welfare of others (*1*). Two reports by Melis *et al*. on page 1297 (*2*) and Warneken and Tomasello on page 1301 (*3*) of this week's issue contribute to understanding how we came to be such caring and cooperative creatures.

Evolutionary theory predicts that altruistic interactions, which are costly to the actor and beneficial to the recipient, will be limited to kin or reciprocating partners. This precludes anonymous acts of altruism on behalf of strangers, such as giving blood, or large-scale cooperation, such as serving on committees. Cooperation is equally perplexing to economists whose theorems are based on the principle of maximizing profit and self-interest, not concern for the welfare of others. Evolutionary theory and economic models provide a comfortable fit for the behavior of other animals (*4*, *5*), including other highly social and intelligent members of the primate order (*6*), but humans stand out as a puzzling anomaly (*1*).

This raises two questions: Why do humans cooperate so much? And what limits the extent of cooperation in other animals? While evolutionary social scientists struggle with the first question, primatologists are beginning to tackle the second. Much of this work focuses on chimpanzees. Chimpanzees participate in a variety of collective activities in the wild, but we can't say much about the motives underlying cooperation or the factors that prevent them from cooperating more in the wild. So researchers have headed into the laboratory to probe the capacity and motivation for cooperation.

To cooperate effectively, individuals must know what needs to be done and be willing to do it. Experimental efforts to induce nonhuman primates (capuchins, tamarins, and chimpanzees) to work together in joint tasks have met with mixed success. But it is not clear whether collaborative failures occurred because animals didn't understand how to solve the tasks (*7*) or because they were inhibited by the presence of competitors who monopolized the apparatus and appropriated rewards (*8*–*10*).

Work with me. A female chimpanzee fishes for termites while her infant sits on her shoulders. Tolerance during feeding enhances the effectiveness for cooperation in joint tasks involving food rewards.

Two sets of experiments conducted by researchers at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany (*2*, *11*) provide compelling evidence that chimpanzees collaborate effectively under appropriate conditions. In one set of experiments (*11*), bowls of food were attached to a platform outside the testing room. A rope was threaded through the platform so that it could be pulled forward only if two chimpanzees pulled on the ends of the rope at the same time. Pairs of chimpanzees that got along well in other settings quickly learned to solve this task together, but chimpanzees paired with less preferred partners were much less successful. The same apparatus was used in another set of experiments (*2*), but with one chimpanzee placed in the testing room and the other in an adjoining room. The chimpanzee in the testing room could admit the other by removing a key that locked the door between the two rooms. First, Melis and her colleagues manipulated the need for collaboration by varying the distance between the ends of the rope threaded through the platform. A chimp was more likely to recruit an assistant when the rope ends were too far apart to be pulled at the same time by one individual. Second, the chimps were allowed to choose between two potential collaborators who differed in their effectiveness in the task. Initially, the chimpanzees did not discriminate between the two assistants, but they came to show a strong preference for the more effective helper.

Both of these experiments indicate that chimpanzees can work together effectively when they profit directly. But humans also provide help when they don't benefit themselves. Warneken and Tomasello suggest that human helpfulness emerges at infancy. The authors presented 18-month-old children with situations in which an adult was trying to perform an everyday task (e.g., reaching for a marker or stacking books). In control trials, no help was needed by the adult. On the majority of tasks, children were more likely to perform the appropriate act (respond to others' needs) when help was needed than in the control condition, and they did so without prompting. These data complement findings that by 15 months of age, infants have some understanding of others' mental states (*12*) and respond to others' distress (*13*).

Warneken and Tomasello also presented three 3- to 4.5-year-old human-reared chimpanzee infants with similar tasks and scenarios. The chimpanzees regularly responded when tasks required reaching, but not in tasks that required other types of assistance, perhaps because they more readily grasped the intended goal in the reaching task than in the other tasks.

Although it is tempting to conclude that the responses of human and chimpanzee infants in these experiments were motivated by empathy, other experiments suggest that chimpanzees are not consistently motivated by concern for the welfare of others. In experiments conducted by two independent research groups at three different research facilities, adult chimpanzees were offered the opportunity to provide rewards to others at no cost to themselves (*14*, *15*). One chimpanzee (the actor) was offered a choice between two options: One option (A) delivered a food reward only to the actor, and the other (B) delivered a reward to the actor, as well as to a familiar group member. Experimenters also included a control condition in which the actor was offered the same options when no other chimpanzee was present. If chimpanzees were concerned about the welfare of others, they would prefer option B. If chimpanzees were indifferent about the welfare of others, they would choose between the two options at random. In both studies, actors were just as likely to

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choose option B when they were alone as they were when another chimpanzee was present.

The chimpanzees' responses in these two sets of experiments were equivalent to flipping a coin to decide whether to toss out that extra copy of *Science*. Human children behave quite differently. When 3- to 5-year-olds were offered a choice between a sticker for themselves and a sticker for the experimenter, or just one sticker for themselves, they overwhelmingly chose the prosocial option (*16*).

It's not clear why chimpanzee infants were helpful to humans, but older chimpanzees did not help other chimpanzees obtain food rewards even when there was no cost in doing so. These studies will no doubt fuel debate about which best captures the essence of chimpanzee cooperation. We can hope that the creative approach of the Leipzig research teams will inspire new experiments to address the arguments.

References

1. E. Fehr, U. Fischbacher, *Nature* **425**, 785 (2003).
2. A. P. Melis, B. Hare, M. Tomasello, *Science* **311**, 1297 (2006).
3. F. Warneken, M. Tomasello, *Science* **311**, 1301 (2006).
4. S. T. Emlen, in *Behavioural Ecology: An Evolutionary Approach*, J. R. Krebs, N. B. Davies, Eds. (Blackwell Scientific, Oxford, 1997), pp. 228–253.
5. L. A. Dugatkin, *Cooperation Among Animals* (Oxford Univ. Press, Oxford, 1997).
6. P. M. Kappeler, C. P. van Schaik, Eds., *Cooperation in Primates and Humans* (Springer, Berlin, 2006).
7. E. Visalberghi, B. P. Quarantotti, F. Tranchida, *J. Comp. Psychol.* **114**, 297 (2000)
8. F. B. M. de Waal, J. M. Davis, *Neuropsychologia* **41**, 221 (2003).
9. K. A. Cronin, A. V. Kurian, C. T. Snowdon, *Anim. Behav.* **69**, 133 (2005)
10. D. Werdenich, L. Huber, *Anim. Behav.* **64**, 771 (2002).
11. A. P. Melis, B. Hare, M. Tomasello, *Anim. Behav.*, in press.
12. K. H. Onishi, R. Baillargeon, *Science* **308**, 255 (2005).
13. C. Zahn-Waxler, M. Radke-Yarrow, E. Wagner, M. Chapman, *Dev. Psychol.* **28**, 126 (1992).
14. J. B. Silk *et al.*, *Nature* **437**, 1357 (2005).
15. K. Jensen, B. Hare, J. Call, M. Tomasello, *Proc. R. Soc. London Ser. B*, in press., published online 17 January 2006 (10.1098/rspb.2005.3417).
16. C. Thompson, J. Barresi, C. Moore, *Cogn. Dev.* **12**, 199 (1997).

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ANTHROPOLOGY

An Example of Preclassic Mayan Writing?

Archaeological discoveries at San Bartolo, Guatemala, have provided a rich trove of information about Maya culture. Painted hieroglyphs in an early temple at this site suggest that Mayan writing had developed by 200 to 300 B.C.

Stephen D. Houston

San Bartolo, in remote northeastern Guatemala, has been the site of several stunning discoveries from ancient Maya civilization. The news agency Notimex (*1*), however, insists that the finds represent a modest discovery, a product of media "diffusion and financial support" from "foreign money." Most scholars would politely disagree. With the San Bartolo murals, and the text now reported by Saturno *et al*. on page 1281 of this issue (*2*), Maya archaeology of Mexico and northern Central America enters a period of renewed focus on the mental and religious life of the late Preclassic period, a time from ~300 B.C. to 100 A.D. As a scientific discipline, the field will be marked by a time before the discovery of these paintings in the jungle of Guatemala, and a time thereafter.

For much of the 20th century scholars have known that the Preclassic was a time of monumental construction, immense stucco masks, and fragmentary remains of wall paintings. But, to quote Winston Churchill in another circumstance, the period offered a riddle wrapped in a mystery inside an enigma. The historical and dynastic detail of the later, Classic period, from ~250 to 850 A.D., seemed undetectable, with the courtly life of the Classic invisible or inferred at best. This has not changed. The shimmer of kings and their doings remains hazy for much of the Preclassic period. What the San Bartolo paintings do is to highlight as never before the inventory of godly narrative from a remote time (see the figure). They confirm the resilience of those ideas over more than a millennium.

The excavation of a small text, in deposits securely dated to 200 to 300 B.C., thrusts San Bartolo into another kind of prominence. As pointed out by Saturno and his coauthors (*2*), the painted block with 10 hieroglyphs forms part of a longer sequence, perhaps in pieces still waiting in this layer behind the mural room. Their content is, as with many Preclassic texts in Mesoamerica, hard to discern. There may be a glyph for "lord," another for "scribe," with a human hand clutching a brush, even a "split sky" sign that resembles later dynastic titles for the kingdoms of El Zotz, Guatemala, and Yaxchilan, Mexico. Yet these are speculative identifications. The authors are correct to stress the opaque nature of early Maya writing and the San Bartolo block. In fact, the opacity itself poses a question: Why is Preclassic script so discontinuous with later, more legible inscriptions? One answer might be that collapse of Preclassic society in the second century A.D. ruptured scribal training along with other features of ancient society. A growing theme in research is the perception that the Maya writing experienced multiple shifts, to the extent that it is best viewed as a writing tradition or a set of historical practices. Maya script is not a unitary system of writing that remains fixed from earliest times.

As a result, the text from San Bartolo commands attention less because of what it records, than because of its striking date and sophistication. The glyphs are hardly the work of a neophyte or an inventive genius from antiquity. The sure execution and balancing of brush width indicate several centuries of prior development, suggesting a set of evidence that awaits discov-

Maya murals. Reproduction of accession scene, West Wall, San Bartolo Mural Building. The standing figure to the left offers a crown and headdress to a seated lord on a painted, wooden scaffold. The undeciphered glyphs at the center may refer to this act.

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ery, provided that excavations target likely places for finds. The new find comes, by the authors' admission, from a comparatively small site. If these discoveries occur in such a place, what can be expected of the gigantic cities of similar date in the Mirador Basin to the north of Guatemala, especially the sites of El Mirador, Wakna, and others? San Bartolo needs more research. At the same time, scholars should turn with refocused energy to the cities that served as the principal audience for—and, maybe, main creators of—this mode of communication. An analogy would be that San Bartolo looks like a small Umbrian chapel of the Renaissance, its murals dedicated, perhaps, to intimate acts of worship or the instruction of religious mysteries to youths and other inductees. Where, though, is the Maya Sistine Chapel?

Even more important, the San Bartolo text relates directly to theories about the origins of writing (*3*). A common approach to this process is "gradualist," meaning that some scholars perceive scripts to develop slowly over long units of time. An opposed tendency is to support a "eureka" instant, rather like Archimedes leaping from the bathtub in joyous insight. If there is scholarly consensus, it is to disfavor the gradualist view and to situate the origins of writing within rapid bursts, in a pattern that roughly resembles the punctuated equilibria of evolutionary biologists. In such episodes, moments of relative morphological stasis follow abrupt change. These changes are often difficult to chart within existing chronological frameworks. Sampling is inadequate to resolve the fine-tuning of time, a predicament highlighted by the surprising nature of the San Bartolo dates. The text cannot be unique. There must have been many such glyphs at other Preclassic sites in Belize, Guatemala, and Mexico. In fact, a smeared guideline under the San Bartolo glyphs implies a more elaborate composition, with other glyphs and scenes yet to be found at this ruin.

The dating of the San Bartolo text leads to a final question. Archaeological journalists often focus on superlatives, stressing the earliest, the biggest, the best. These claims make professionals roll their eyes but raise a good point. The San Bartolo block achieves importance because it narrows the time in which writing first appears in Mesoamerica. Most early finds, regardless of region, now come close to the midpoint of the first millennium B.C. There is little doubt that writing arose from the codified imagery of the Olmec style, widely diffused in coherent form across much of Mesoamerica. With San Bartolo and other discoveries, the beginnings of writing appear now to lie within a relatively short span, over a wide area. The challenge of future research is to explain that singular burst of creativity—to find, not Archimedes, but the handiwork of his Mesoamerican counterparts.

Reference

1. http://srv2.vanguardia.com.mx/hub.cfm/FuseAction.Detalle/Nota.503906/SecID.29/index.sal
2. W. A. Saturno, D. Stuart, B. Beltrán, *Science* **311**, xxx (2006); published online 5 January 2006 (10.1126/science.1121745).
3. S. Houston, Ed., *The First Writing: Script Invention as History and Process* (Cambridge Univ. Press, Cambridge, UK, 2004).

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PLANETARY SCIENCE

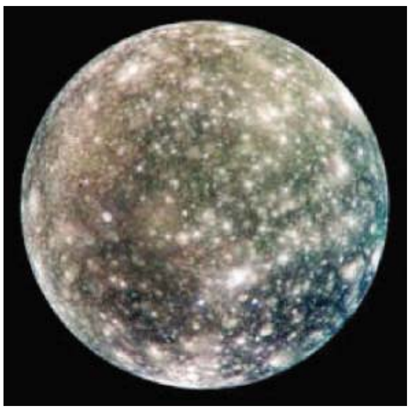
Creep and Flow on the Icy Moons of the Outer Planets

On Earth, the flow of ice depends on its grain size. Higher pressure forms of ice such as those within the icy moons of the outer planets behave similarly, significantly affecting models of their internal dynamics.

Peter R. Sammonds

On Earth, ice in glaciers and ice sheets can flow superplastically (that is, this ice can deform much more than the normal range for a given stress). This is possible because of time-dependent creep that is sensitive to the grain size of the ice (*1*). This recent realization, although controversial (*2*), has deeply influenced glaciological thinking and has led to models that could better explain modern and ancient ice sheet behavior (*3*). Now Kubo and his colleagues report on page 1267 of this issue (*4*) that grain size–sensitive creep occurs in a high-pressure water ice that is a major constituent of the moons of the outer solar system. This realization could change our understanding of the dynamics and evolution of these planetary bodies (see the figure).

The dynamics of glaciers and ice sheets on Earth are largely controlled by grain-scale deformation of ice (*1*). Naturally occurring ice on Earth, Ice I, creeps along at low stresses by solid-state viscous flow. Microscopically, what is happening is that line defects in the crystal lattice, called dislocations, glide within the ice grains as carriers of deformation, enabling the ice bulk to creep along in time. This mechanism of deformation is called dislocation creep. Because the deformation is occurring within individual ice grains, dislocation creep does not depend on the size of the ice grains; it is grain size–insensitive. However, Goldsby and Kohlstedt (*1*) showed in laboratory experiments that for very fine-grained Ice I deforming at very low stresses, ice deformation does depend on grain size; it is grain size–sensitive. Under these conditions, grain boundary sliding accommodates deformation as ice grains slide past each other. Ice deforming under these low stresses is orders of magnitude less viscous than at high stresses. They described this as superplasticity in ice. At still lower stresses, ice can deform by defects diffusing through the ice lattice and along grain boundaries; this is called diffusional flow, which is also grain size–sensitive.



Grain size matters. Ice flow that depends on grain size may dominate the dynamics and evolution of the icy moons. This full-color image of Jupiter's icy moon Callisto was taken by the Galileo orbiter in May 2001.

CREDIT: NASA

On Earth, Ice I, as Poirier (*5*) pointed out, is a unique rock-forming mineral in that it is the only one that is so close to the solid-liquid-vapor triple point at the temperatures and pressures of Earth's surface. Ice I has a crystal lattice structure of stacked puckered hexagonal rings of oxygen ions with a disordered hydrogen ion (proton) sublattice. On moons of the outer solar system such as Jupiter's Europa, Ganymede, and Callisto and Neptune's Triton, water ice and its high-pressure polymorphs are also important rock-forming minerals and major constituents of these moons. Although the outer skins of the icy moons would be the low-pressure Ice I, in the interior the high-pressure polymorphs could be

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present. At high pressures and low temperatures, Ice I transforms to Ice II, which has a rhombohedral crystal lattice structure of hexagonal tubes of oxygen ions with an ordered proton sublattice. Higher pressure ice polymorphs Ices V, VI, and VII may also be present in the interiors of the larger moons.

What Kubo and his colleagues have now shown is that a high-pressure polymorph of ice, Ice II, also deforms by grain size–sensitive creep at low stresses. They have done this by fabricating fine-grained Ice II by transformation from Ice I in a cryogenic high-pressure cell and then deforming the ice in the cell to measure the flow stress at which it creeps. By cycling an Ice II sample back to Ice I and then transforming it to Ice II again, they created still finer grained Ice II, measured its flow stress, and so on. They found that triply transformed Ice II flows at less than half the stress of a single-transformation sample. The grain sizes of their singly, doubly, and triply transformed samples were revealed by scanning electron microscope (SEM) analyses of the indium metal sleeves that jacket the ice samples, as well as by direct cryogenic SEM analyses of Ice II grains partially decorated with Ice I. They found a correlation between the number of transformation cycles and the flow stresses, demonstrating that the rheology of Ice II is grain size–sensitive at low stresses.

This finding has direct planetary implications because stress levels are low in the convecting interiors of the icy moons. Kubo *et al*. argue that grain size–sensitive creep of Ice I and Ice II plausibly dominates the evolution and dynamics of the interiors of the medium to large icy moons of the outer solar system. Ice II is considerably more viscous than Ice I. The transition from Ice I to Ice II, which occurs at depth, is accompanied by an increase in viscosity of four orders of magnitude. If grain size–sensitive creep does not operate, then the increase in viscosity would be six orders of magnitude. So if grain size–sensitive creep is not taken into account as a deformation mechanism, estimates for viscosities of the interiors of the icy moons are off by about two orders of magnitude. Such a difference would have profound implications for interpreting their evolution and dynamics. But this also has implications for the theoretical and experimental research now needed. Modeling of planetary dynamics cannot be based solely on mineral physics calculations of single-crystal behavior, nor on experiments on single crystals of ice. We need to understand the bulk physical and mechanical behavior of the rocks formed from ice and its high-pressure polymorphs in order to better understand the icy moons of the outer solar system.

References

1. D. L. Goldsby, D. L. Kohlstedt, *J. Geophys. Res*. **106**, 11017 (2001).
2. P. Duval, M. Montagnat, *J. Geophys. Res.* **107**, 10.1029/2001JB000946 (2002).
3. W. R. Peltier, D. L. Goldsby, D. L. Kohlstedt, L. Tarasov, *Ann. Glaciol.* **30**, 163 (2000).
4. T. Kubo, W. B. Durham, L. A. Stern, S. H. Kirby, *Science* **311**, 1267 (2006).
5. J. P. Poirier, *Nature* **299**, 683 (1982).

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STRUCTURAL BIOLOGY

Architectural Options for a Fatty Acid Synthase

Multiple enzymes produce fatty acids in cells. These enzymes assemble into large complexes that are quite different in animals and fungi, but still carry out the same chemical synthesis.

Stuart Smith

The constituent enzymes of conserved biosynthetic processes do not necessarily assume the same organization across species. Take the enzymes required for the de novo biosynthesis of fatty acids, important cellular energy storage and structural molecules. In prokaryotes, the enzymes are freestanding individual proteins, whereas in eukaryotes, they are covalently linked into large, multifunctional polypeptides. Surprisingly, in eukaryotes, evolution of fatty acid synthases has proceeded along entirely different lines, resulting in two distinct architectural forms: a 2.6-megadalton barrel-shaped structure in fungi and a 0.54-megadalton X-shaped structure in animals (see the figure).

The animal fatty acid synthase plays an essential role in embryogenesis and energy homeostasis and is a target for the development of both anti-obesity and anti-cancer agents. Furthermore, it has served as a useful paradigm for understanding the structural and functional organization of multimodular enzymatic assembly lines that synthesize polyketides, important pharmacological agents. These megasynthases can contain multiple fatty acid synthase–like functional modules on a single polypeptide chain. Despite interest in fatty acid synthases, structural information has been limited to low-resolution (16 to 21 Å) electron micrographic reconstructions which, regarding animal forms, have been interpreted to support two quite different models. Crystals of both eukaryotic synthases were obtained several decades ago, but had not been exploited for structural analysis. This impasse finally has been overcome by Ban and colleagues. On page 1263 and 1258 in this issue, they report the successful application of x-ray crystallography to derive electron density maps for both a fungal (*1*) and a mammalian fatty acid synthase (*2*) to a resolution of ~5 Å. This breakthrough provides new insights into the architecture of these megasynthases and resolves the ongoing controversy over the animal enzyme structure.

Although the resolution is insufficient to identify amino acid side chains, or trace the complete backbone of individual subunits, the authors have fit three-dimensional structures of homologous individual bacterial proteins into the electron density maps of both synthases to reveal the location of most of the functional domains. The 5 Å structures agree well with the lower resolution electron micrographic structures (*3*, *4*). In both crystal structures, electron density that could not be assigned to catalytic domains may represent interdomain regions that stabilize these large oligomeric complexes. The location of the acyl carrier protein (ACP) domain that carries reaction intermediates to the various catalytic centers also could not be unambiguously assigned in either structure, presumably because of its inherent mobility.

The fungal fatty acid synthase is a barrel-shaped dodecamer constructed from two non-identical polypeptides ($\alpha_6\beta_6$). As predicted from earlier studies, the perimeter of the equatorial region of the barrel is derived from the α chains and the sides of the barrel from the β-chains. The barrel is divided by a central wheel-like structure into two reaction chambers. The equatorial region consists of three alternating β-ketoacyl synthase (KS) and β-ketoacyl reductase (KR) homodimers, with one active site of each dimer directed toward either the upper or lower chamber. Four monomeric domains that constitute the barrel's sides have active centers directed toward the interior. The authors speculate that the six ACP domains are tethered to the central wheel, with three directed toward each of the two chambers. Thus, each chamber contains three copies of all of the functional domains with interior-facing active sites. Small pores in the sides of the barrel allow diffusion of

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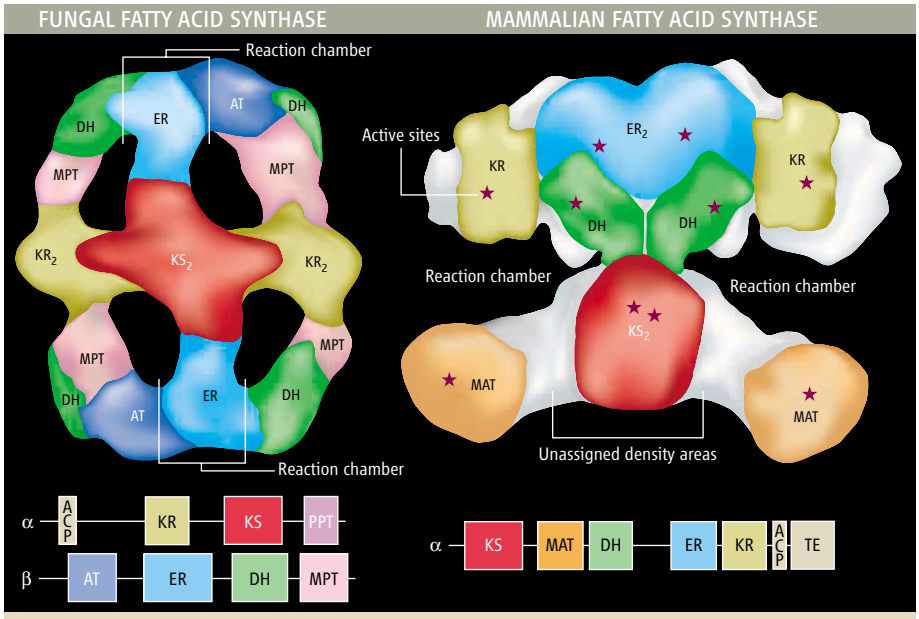
the substrates acetyl and malonyl–coenzyme A (CoA) into the reaction chambers and exiting of the palmitoyl-CoA product (*3*).

The original model for the animal fatty acid synthase, formulated in the 1980s, envisioned two subunits (α_2) orientated in a fully extended antiparallel arrangement in which a noncatalytic "central core" stabilized a dimeric structure. Despite evidence questioning its validity, this model still enjoys some support (*5*, *6*). Its proponents attempted to fit an x-ray crystal structure for the thioesterase (TE) domain and a modeled monomeric KS domain into a low-resolution electron micrographic structure but, as revealed by the new 5 Å resolution structure, both domains were incorrectly positioned (*7*). This outcome illustrates the perils of this approach. Although it would have been more reassuring if Ban and colleagues had provided some objective assessment of the "goodness of fit" of the homologous crystal structures into their electron density maps, the 5 Å resolution of their structure strongly suggests that they have got it right. Indeed, their structure agrees well with a model derived from mutant complementation (*8*), cross-linking (*9*), and electron micrographic (*4*) experiments. The new model depicts two coiled subunits oriented head to head, with paired KS domains stabilizing the dimer (*10*). The crystal structure confirms the dimeric nature of the KS domains but also reveals some surprises. The β-enoyl reductase (ER) domains are also dimeric and the dehydratase (DH) domains appear pseudodimeric, with each pseudosubunit coming from adjacent regions of the same polypeptide. Furthermore, the central core of the synthase appears to be interspersed along the "arms" of the structure.

The animal fatty acid synthase can be conveniently characterized as comprising a "body" (the ER and KS dimers and pseudodimeric DH pairs) with two "arms" (KR monomers) and "legs" [monomeric malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT) domains]. The ACP and TE domains which, because of inherent mobility, could not be assigned unambiguously to the electron density map, most likely are located at the end of the two arms, by virtue of their location in the primary sequence, adjacent to the KR domains. Active sites of the two sets of catalytic domains are oriented facing each of the two lateral clefts in the structure, thus forming the two chambers. Interestingly, these reaction chambers do not appear in identical conformations; the distances between active sites associated with the arms and legs are different and blurred electron density, possibly attributable to the ACP and/or TE domains, is visible only on one side of the structure. It has been suggested that the synthesis of fatty acids at the two sites may function asynchronously (*4*), with one chamber engaged in carbon-chain elongation and the other in β-carbon processing. By superimposing the two sides of the synthase dimer on each other, Ban and colleagues have cleverly exploited this lateral asymmetry to reveal hinge regions that may facilitate the adoption of different conformations by this enzyme.



Distinct organization of two eukaryotic fatty acid synthases. (**Left**) Fungal fatty acid synthase assumes a barrel-like shape (260 Å high, 230 Å wide). (**Right**) Mammalian (porcine) fatty acid synthase is an asymmetric X-shape (210 Å high, 180 Å wide, 90 Å deep). Side views are shown. In the fungal enzyme, acetyl transferase loads the acetyl primer substrate whereas the malonyl/palmitoyl transferase loads malonyl moieties and releases the palmitoyl-CoA product. The animal synthase loads both substrates via the malonyl-CoA-/Acetyl CoA-ACP transacylase and unloads free palmitic acids via a thioesterase. The acyl carrier protein of the fungal synthase is posttranslationally modified by phophopantetheinyl transferases that are likely localized as timers at the barrel apices.

What new insights may be gleaned from the animal fatty acid synthase structure that might be applicable to other megasynthases? One surprising aspect of the animal structure is the substantial intersubunit contacts between the pairs of ER and DH domains along the pseudo-twofold axis of symmetry. Many modules associated with polyketide synthases completely lack these enzymatic domains that are required for β-carbon processing reactions. In addition, whereas the TE domains of the animal fatty acid synthase are monomeric, those associated with the terminal modules of polyketide synthases are dimeric. These considerations indicate that, despite similarities in the ordering of their component domains, the synthases involved in fatty acid and polyketide production may rely on different interactions to stabilize their complex architecture. It is hoped that the successful application of crystallographic analysis to the fatty acid synthase system will spark interest in developing high-resolution structures for the modular polyketide synthases and reveal how multiple modules are linked together to form an enzymatic assembly line.

References

1. S. Jenni, M. Leibundgut, T. Maier, N. Ban, *Science* **311**, 1263 (2006).
2. T. Maier, S. Jenni, N. Ban, *Science* **311**, 1258 (2006).
3. S. J. Kolodziej, P. A. Penczek, J. P. Schroeter, J. K. Stoops, *J. Biol. Chem.* **271**, 28422 (1996).
4. F. J. Asturias *et al.*, *Nat. Struct. Mol. Biol.* **12**, 225 (2005).
5. S. S. Chirala, A. Jayakumar, Z. W. Gu, S. J. Wakil, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3104 (2001).
6. N. Kresge, R. Simoni, R. L. Hill, *J. Biol. Chem.* **281**, e5 (2006).
7. B. Chakravarty, Z. Gu, S. S. Chirala, S. J. Wakil, F. A. Quiocho, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15567 (2004).
8. V. S. Rangan, A. K. Joshi, S. Smith, *Biochemistry* **40**, 10792 (2001).
9. A. Witkowski *et al.*, *J. Biol. Chem.* **274**, 11557 (1999).
10. A. Witkowski *et al.*, *Chem. Biol.* **11**, 1667 (2004).
11. K. Werkmeister, R. B. Johnston, E. Schweizer, *Eur. J. Biochem.* **116**, 303 (1981).

10.1126/science.1125411

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Auxiliary Subunits Assist AMPA-Type Glutamate Receptors

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Glutamate, the major excitatory neurotransmitter in the brain, acts primarily on two types of ionotropic receptors: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and *N*-methyl-D-aspartate (NMDA) receptors. Work over the past decade indicates that regulated changes in the number of synaptic AMPA receptors may serve as a mechanism for information storage. Recent studies demonstrate that a family of small transmembrane AMPA receptor regulatory proteins (TARPs) controls both AMPA receptor trafficking and channel gating. TARPs provide the first example of auxiliary subunits of ionotropic receptors. Here we review the pivotal role that TARPs play in the life cycle of AMPA receptors.

"Life is all memory, except for the one present moment that goes by you so quick you hardly catch it going." This statement by Mrs. Goforth in *The Milk Train Doesn't Stop Here Anymore*, by Tennessee Williams, succinctly addresses what the brain does. We are little more than the compilation of our memories, and these memories make each of us unique. Thus, understanding how the brain acquires and stores information is one of the foremost challenges in neurobiology.

For more than a century, activity-dependent changes in synaptic strength have been postulated as critical for learning and memory. The discovery of long-term potentiation (LTP) of excitatory synapses in the hippocampus (*1*), a brain structure essential for certain forms of memory, provided the first decisive evidence; LTP remains intensively studied. Excitatory synapses release glutamate onto two types of ionotropic receptors, AMPA receptors (AMPARs) and NMDA receptors (NMDARs). Whereas at least two mechanistically distinct forms of LTP exist (*2*), the most widespread form requires the activation of NMDARs, augmentation of postsynaptic calcium, and the activation of the calcium/calmodulin dependent kinase II (CaMKII) (*3*–*7*).

The mechanisms underlying the changes that occur during LTP have been difficult to define (*8*). The discovery of silent synapses and the evidence that LTP unsilences these synapses (*9*, *10*) have convinced most researchers that LTP involves the activity-dependent rapid recruitment of synaptic AMPARs. Direct support comes from physiologically tagged AMPAR protein subunits (*11*–*13*) and experimentally uncaging glutamate onto single spines (*14*, *15*).

AMPARs are heterotetramers comprising combinations of glutamate receptors 1 to 4 (GluR1–4) subunits. In hippocampal pyramidal cells, AMPARs primarily comprise either GluR1/2 or GluR2/3. Synaptic trafficking of AMPARs depends on their subunit composition; GluR2/3 receptors constitutively cycle into and out of the synapse, whereas the trafficking of GluR1/2 receptors require activity (*12*).

Stargazer Mice

To understand receptor trafficking, investigators have defined interactions of the cytoplasmic tails of GluR1–4 with cytosolic proteins such as GRIP/ABP (glutamate receptor–interacting protein/AMPA receptor–binding protein), PICK1 (protein interacting with C kinase), and NSF (N-ethylmaleimide–sensitive factor) (*12*, *16*–*22*). Another approach has been the screening of mutant mice with well-defined motor defects. The stargazer mouse is both ataxic and epileptic. This mouse selectively lacks functional AMPARs in cerebellar granule cells (Fig. 1, A and B) (*23*, *24*). The mutated protein, stargazin (also known as γ-2), is a small tetraspanning membrane protein with some homology to a calcium channel subunit γ-1 (*25*). Transfecting stargazin into cultured cerebellar granule neurons from stargazer mice restores both synaptic and extrasynaptic AMPAR responses (Fig. 1, C to E) (*26*). Stargazin shares homology with a large family of proteins (*27*, *28*), and a subset of four (γ-2, γ-3, γ-4, and γ-8) can also traffic AMPARs (*29*). These four transmembrane AMPAR regulatory proteins (TARPs) (*29*) are differentially expressed throughout the brain.

Stargazin Binds to AMPARs

The immunoprecipitation of brain extracts shows that TARPs robustly and uniquely interact with all AMPAR subunits (*29*–*31*). Prolonged incubation of these immunoprecipitates with high concentrations of glutamate, which may occur during excitotoxicity, causes stargazin and AMPARs to dissociate (*30*).

Separation of cerebellar extracts by native polyacrylamide gel electrophoresis revealed two populations of AMPAR complexes, and stargazin comigrated with the larger complex (*32*). The higher molecular weight AMPAR complex was absent in extracts from stargazer cerebellum. This suggests that the higher weight AMPAR complex consists of the tetrameric receptor bound to stargazin, whereas the lower weight form represents apo-AMPARs. Structural analyses of purified AMPARs at ~40 Å resolution show that TARPs contribute to the density representing the transmembrane region of the forebrain AMPAR complex (*33*). These biochemical studies establish that TARPs bind selectively and stoichiometrically to AMPARs. However, the number of TARPs in a tetrametric AMPAR complex remains uncertain. A number of cytosolic proteins have been reported to bind to the C-terminal tails of AMPAR subunits (*6*, *7*), and it will be important to find conditions that preserve these interactions in brain extracts.

AMPAR Maturation Requires Stargazin

The amount of GluR2 protein in the cerebellum, which is prominently expressed in granule cells, is reduced by about 20% in the stargazer mouse. Membrane proteins undergo a regulated biosynthetic progression through the endoplasmic reticulum (ER) and Golgi. Glutamate receptors receive high mannose glycosylation in the ER and are later modified with more complex sugars in the Golgi. The GluR2 protein that remains in the stargazer cerebellum has an immature ER-type glycosylation, suggesting that stargazin traffics AMPARs early in the biosynthetic pathway (*29*). Further evidence that TARPs stabilize AMPAR proteins is that the deletion of stargazin induces an ER unfolded-protein response in cerebellar granule cells (*34*).

Stargazin Traffics AMPARs by Two Mechanisms

Why do stargazer cerebellar granule cells lack synaptic AMPARs? First, AMPAR translocation from an intracellular site to the cell surface requires stargazin (*26*). In the *Xenopus* oocyte system (*35*–*37*), stargazin enhances the surface expression of all AMPAR subunit combinations. By contrast, stargazin does not traffic closely related kainate receptors (*35*). Second, the last four amino acids of stargazin bind to the PDZ domains of a number of synaptic scaffolding proteins, including PSD-95 (*26*). This PDZ interaction mediates synaptic targeting of surface receptors. Thus, transfecting a stargazin construct lacking the last four amino acids

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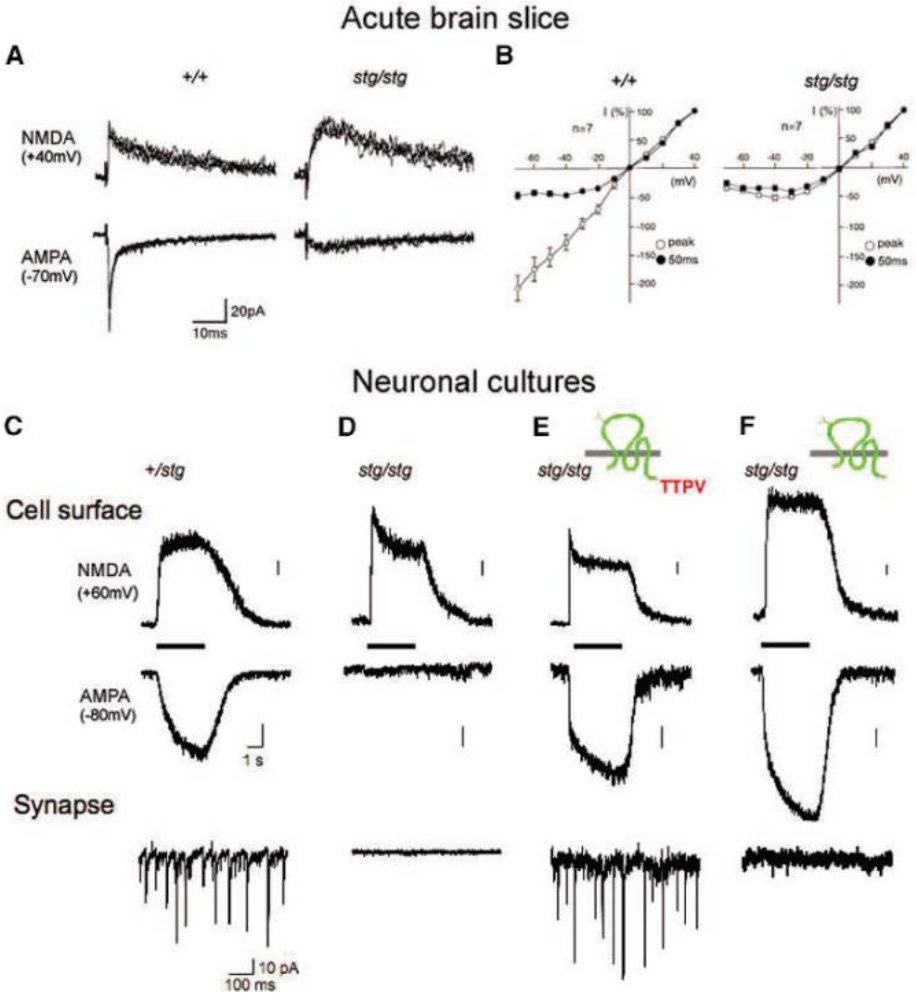

Fig. 1. Cell surface and synaptic AMPAR responses in stargazer (*stg/stg*) cerebellar granule cells require stargazin and stargazin C-terminal PDZ binding sites, respectively. (**A**) EPSCs elicited by mossy-fiber (MF) stimulation in granule cells from the wild-type (+/+) and *stg* mutant (*stg/stg*) mice at holding potentials of +40 mV (top panels), which records primarily the NMDA EPSC, and −70 mV (bottom panels), which records primarily the AMPA EPSC. Each trace is a single-sweep record, and several traces are superimposed for each record. (**B**) Current-voltage (*I-V*) relationships of MF-EPSCs from wild-type and *stg* mutant mice measured at the peak (open circle) and 50 ms after (closed circle) the stimulus. The EPSC amplitudes were normalized to the mean value at −40 mV in each experimental condition. Each data point and attached error bar represent mean and SEM. (A) and (B) are from (*24*). (**C** to **F**) Examples of glutamate-evoked (100 μM) whole-cell currents recorded at −80 mV, which evokes primarily AMPAR responses, and at +60 mV, which evokes primarily NMDA responses. The perfusion solution contained tetrodotoxin (1 μM) and glycine (20 μM). At −80 mV, glutamate evoked an inward current in a +/stg neuron (C), which is absent in stg/stg neurons (D). Stargazin rescues AMPAR responses and synaptic AMPAR responses in stg/stg granule cells (E). StargazinΔC rescues AMPAR responses but not synaptic AMPAR responses in stg/stg granule cells (F). Calibration bars are, from left to right, 25, 50, 25, and 25 pA (*y* axes), and 1 s (*x* axis) (top) and 25, 50, 50, and 25 pA, and 1 s (bottom). The calibration is the same for all synaptic responses.

(stargazinΔC) into stargazer granule cells rescues surface, but not synaptic, AMPARs (Fig. 1F). Furthermore, transfecting this construct into wild-type granule cells reduces synaptic AMPAR responses, presumably because of a dominant negative effect (*26*).

Although synaptic transmission in the stargazer hippocampus is normal, stargazin transfections have dramatic effects in hippocampal neurons (*38*). The overexpression of stargazin increases the number of extrasynaptic AMPARs but has no effect on AMPAR-mediated synaptic transmission. By contrast, transfecting stagazinΔC selectively reduces synaptic AMPAR transmission. Overexpression of PSD-95 enhances AMPAR-mediated synaptic responses (*38*–*41*). Because PSD-95 does not bind to AMPAR subunits, it seems that stargazin, which binds both to AMPARs and to PSD-95, mediates AMPAR enhancement by PSD-95 (*38*). These results suggest that the interaction of PSD-95 or related PDZ proteins with TARPs participates in the synaptic targeting of AMPARs in many neuronal types (Fig. 2).

Stargazin Determines Gating of Native AMPARs

Initial studies suggested that all stargazin effects involve receptor trafficking. However, recent studies combining biochemical and biophysical approaches revealed that the enhancement of AMPAR currents by stargazin exceeds the increase in number of surface receptors (*36*, *37*, *42*). Thus, stargazin also enhances the function of the AMPARs.

Stargazin increases the apparent affinity of AMPARs for glutamate (*36*, *37*, *42*). Stargazin causes a four- to sixfold increase of steady-state responses and slows the rate of desensitization and deactivation twofold (*36*, *37*, *43*). Furthermore, stargazin enhances the single-channel conductance of AMPAR (*36*). Analysis of neuronal AMPAR single channels reveals that they can open to four conductance levels. When AMPARs are heterologously expressed alone, most openings are to low-conductance levels, whereas coexpression with stargazin increases the prevalence of high-conductance openings. All of these effects on AMPAR properties would be expected to enhance AMPAR synaptic responses.

Stargazin also modulates AMPAR gating by pharmacological agents (*36*, *43*). Kainate is a partial agonist when AMPARs are expressed alone, but kainate becomes a full agonist with stargazin. Structural studies of the isolated GluR ligand-binding domain indicate that agonists induce closure of the clamshell-shaped binding pocket and that this movement gates the channel. The extent of domain closure is greater for the full agonist glutamate than for the partial agonist kainate (*44*) or for other partial agonists (*45*). These findings predict that the degree of domain closure with glutamate, and especially with kainate, is enhanced by stargazin (Fig. 3).

Are these findings applicable to native receptors? A systematic study of GluR subunit combinations that are likely to be present in cerebellar granule cells (*46*) failed to find a combination that showed the high-conductance openings to glutamate and kainate found in cerebellar granule cells (*47*, *48*). By contrast, coexpression of GluR4, a prominent GluR subunit in cerebellar granule cells, and stargazin generated channels showing the neuronal-type high-conductance openings (*36*).

Are the size and kinetics of synaptic receptors governed by TARPs? This was addressed with chimeras of stargazin and γ-5, a related protein that does not affect AMPARs (*36*). The stargazin first extracellular loop modulates channel properties, whereas the C terminus mediates receptor trafficking. Thus, a stargazin chimera containing the first extracellular loop of γ-5 (Ex1) trafficked the receptors normally but had no effect on deactivation or desensitization, whereas a chimera

lacking the stargazin C terminus did not traffic AMPAR but did modulate their channel properties. The expression of Ex1 in neurons should act as a dominant negative in terms of the biophysical properties of AMPARs. Indeed, in the presence of the Ex1 mutant, the amplitude of AMPAR excitatory postsynaptic currents (EPSCs) was reduced and the decay kinetics were increased. A rough estimate suggests that stargazin biophysical effects increase the charge transfer of synaptic AMPAR responses by 30%.

TARPs in the Hippocampus

To test whether TARPs may modulate AMPAR throughout the brain, the gene for TARP γ-8, which is highly expressed in the hippocampus, was deleted (*49*). The γ-8 knockout (KO) mice were born in a Mendelian ratio, and they did not differ from littermate controls in terms of weight and gross behavior. However, AMPAR proteins (GluR1 and GluR2) were reduced by 80 to 90% in the hippocampus of the γ-8 KO mice. Immunohistochemistry also showed a profound loss of GluR1 and GluR2 in the hippocampal dendrites, with some GluR1/2 protein remaining in neuronal soma. AMPAR-mediated synaptic transmission was reduced by 35%. The density of extrasynaptic AMPARs, measured electrophysiologically, was reduced by 90%. These observations resemble those found for the GluR1 KO mouse (*50*) and suggest that hippocampal pyramidal cells, when confronted with a limited number of AMPARs, sequester the remaining receptors to synapses. Finally, LTP was greatly reduced, but long-term depression (LTD) was normal in the γ-8 KO mouse.

A number of questions concerning the γ-8 KO mouse remain. For instance, what accounts for the persistent synaptic AMPARs? Are they "TARPless," or are the other TARPs in hippocampal pyramidal cells playing a role? What is the basis for the defect in LTP? Does γ-8 play a direct mechanistic role in LTP, or is the defect due to the loss of extrasynaptic AMPARs that might supply synaptic AMPARs during LTP?

Stargazin and Synaptic Plasticity

Because protein kinases play a central role in LTP induction, TARP phosphorylation was explored. Phosphopeptide mapping revealed that the C-terminal tail of stargazin has nine phosphorylated serines (*51*). These serines are conserved in all TARPs. Both CaMKII and protein kinase C, which have been implicated in LTP, phosphorylate some of these serines. In addition, these serines are dephosphorylated by the phosphatases PP1 and PP2b, which have been implicated in LTD. To evaluate the role for these serines in LTP induction, we mutated them to alanine, thereby preventing stargazin phosphorylation. This stargazin mutant still traffics AMPARs; however, LTP could not be generated in neurons expressing this construct. As a converse approach, the serines were mutated to aspartic acid to mimic phosphorylation. This construct effectively delivered receptors to the membrane surface, but, in addition, it drove AMPARs to the synapse in an activity-independent manner. Furthermore, neurons expressing this construct did not generate LTD, presumably because stargazin was locked into a phosphomimic state.

Conclusion

Remarkable progress has been made over the last decade in understanding the molecular machines responsible for trafficking AMPARs to excitatory synapses. This progress has been fueled, in large part, by the realization that synaptic plasticity is mainly mediated by changes in the number of synaptic AMPARs. Stargazin and related TARPs have emerged as primary AMPAR auxiliary subunits that control both AMPAR trafficking and channel gating.

A number of issues remain unresolved. Are TARPs required for all AMPAR trafficking in the central nervous system? In heterologous systems, some AMPAR trafficking occurs without TARPs. Does this also occur in neurons? Generating mutant mice lacking all four TARPs can address this. Might AMPARs have other essential subunits? Functional AMPARs in *Caenorhabditis elegans* require suppressor of lurcher 1 (SOL-1) protein, which is structurally unrelated to stargazin (*52*). Whether a SOL-1–like mechanism operates in mammalian neurons remains unclear.

The existence of four TARPs and four AMPAR subunits raises intriguing possibilities. Might TARP subtypes differ in their capacity to traffic AMPARs and to affect channel gating? This may indeed be the case (*36*, *43*). In some neuronal types, AMPARs of different subunit compositions localize to different synapses in the same neuron (*53*). Might TARP subtypes expressed in these neurons differentially traffic the AMPARs? Do other iontropic receptors have

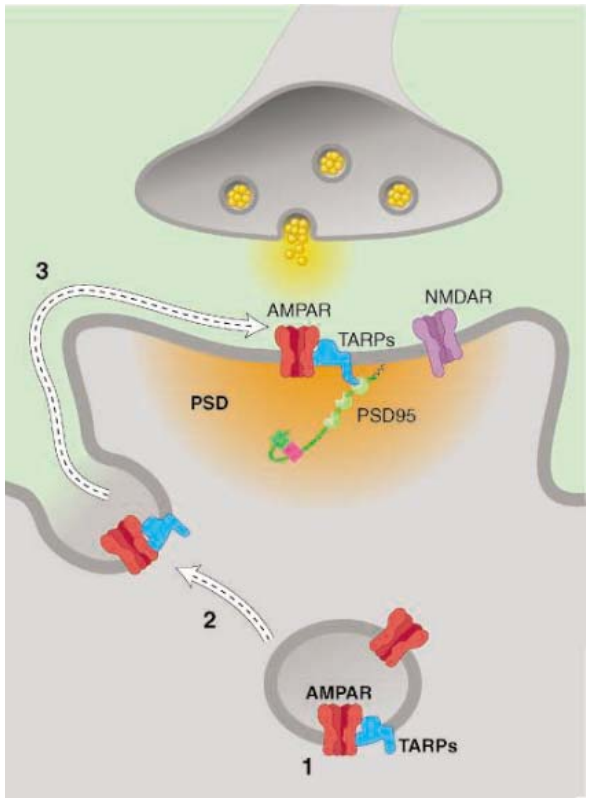

Fig. 2. Roles of stargazin-like TARPs in the trafficking of AMPARs. TARPs (blue) bind to AMPARs (red) early in the synthetic pathway (1) and are required for the trafficking of receptors to the surface (2). Interaction of the C-terminal PDZ binding site of TARPs with PSD-95 at the postsynaptic density (PSD) captures the surface AMPARs at the synapse (3).

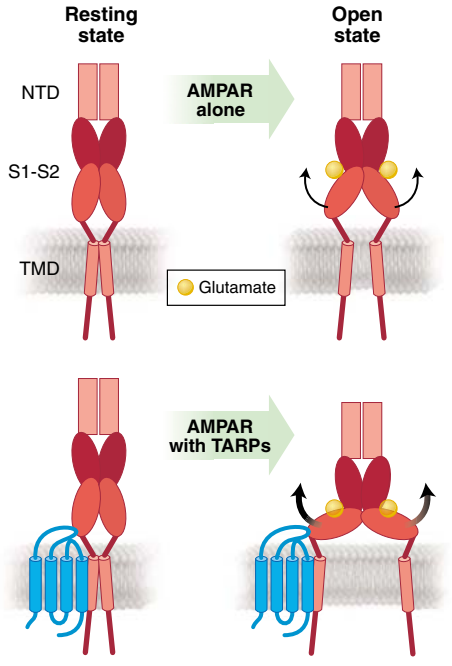

Fig. 3. A model for possible role of TARPs on AMPAR channel opening. AMPAR subunits consist of four domains: a large N-terminal domain (NTD), a ligand-binding pocket (S1-S2), transmembrane domains that form a channel pore (TMD), and a cytoplasmic domain. Upon glutamate binding, S1-S2 closes like a clamshell, which causes channel pore opening. TARPs bind to AMPAR and affect AMPAR channel opening either by inducing more closure of S1-S2–to–glutamate binding or more efficient coupling of domain closure to pore opening without any change in S1-S2 closure.

auxiliary subunits? Because AMPAR trafficking plays a central role in plasticity, perhaps the TARPs evolved to assist in this specialized dynamic mechanism. It has been reported that the cytosolic scaffolding protein PSD-95 can alter the gating of NMDARs (*54*) and the desensitization of kainate receptors (*55*). Another critical question is whether TARP-dependent trafficking shows any GluR subunit specificity. A central challenge will be to determine what role TARPs might have, if any, in the subunit specific control of AMPAR trafficking.

Finally, this Review focused on stargazin and its homologs solely in control of AMPAR function. Do these proteins have other functions? Another possible role is the control of calcium channels, which was originally proposed for these proteins (*25*). It would be intriguing if TARPs could serve as auxiliary subunits for both voltage-gated channels and ionotropic channels. As is the case with any rapidly emerging field, there are many more questions than answers. We can undoubtedly expect many surprises over the next few years.

References and Notes

1. T. V. Bliss, T. Lomo, *J. Physiol.* **232**, 331 (1973).
2. R. A. Nicoll, D. Schmitz, *Nat. Rev. Neurosci.* **6**, 863 (2005).
3. D. S. Bredt, R. A. Nicoll, *Neuron* **40**, 361 (2003).
4. G. L. Collingridge, J. T. Isaac, Y. T. Wang, *Nat. Rev. Neurosci.* **5**, 952 (2004).
5. R. Malinow, R. C. Malenka, *Annu. Rev. Neurosci.* **25**, 103 (2002).
6. M. Sheng, M. J. Kim, *Science* **298**, 776 (2002).
7. I. Song, R. L. Huganir, *Trends Neurosci.* **25**, 578 (2002).
8. R. C. Malenka, R. A. Nicoll, *Science* **285**, 1870 (1999).
9. J. T. Isaac, R. A. Nicoll, R. C. Malenka, *Neuron* **15**, 427 (1995).
10. D. Liao, N. A. Hessler, R. Malinow, *Nature* **375**, 400 (1995).
11. Y. Hayashi *et al.*, *Science* **287**, 2262 (2000).
12. S. Shi, Y. Hayashi, J. A. Esteban, R. Malinow, *Cell* **105**, 331 (2001).
13. S. Q. Liu, S. G. Cull-Candy, *Nature* **405**, 454 (2000).
14. M. Matsuzaki, N. Honkura, G. C. Ellis-Davies, H. Kasai, *Nature* **429**, 761 (2004).
15. A. A. Bagal, J. P. Kao, C. M. Tang, S. M. Thompson, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 14434 (2005).
16. C. Luscher *et al.*, *Neuron* **24**, 649 (1999).
17. A. Luthi *et al.*, *Neuron* **24**, 389 (1999).
18. J. Xia, H. J. Chung, C. Wihler, R. L. Huganir, D. J. Linden, *Neuron* **28**, 499 (2000).
19. S. H. Lee, L. Liu, Y. T. Wang, M. Sheng, *Neuron* **36**, 661 (2002).
20. H. J. Chung, J. P. Steinberg, R. L. Huganir, D. J. Linden, *Science* **300**, 1751 (2003).
21. M. F. Barry, E. B. Ziff, *Curr. Opin. Neurobiol.* **12**, 279 (2002).
22. M. Wyszynski *et al.*, *J. Neurosci.* **19**, 6528 (1999).
23. L. Chen, S. Bao, X. Qiao, R. F. Thompson, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12132 (1999).
24. K. Hashimoto *et al.*, *J. Neurosci.* **19**, 6027 (1999).
25. V. A. Letts *et al.*, *Nat. Genet.* **19**, 340 (1998).
26. L. Chen *et al.*, *Nature* **408**, 936 (2000).
27. D. L. Burgess, L. A. Gefrides, P. J. Foreman, J. L. Noebels, *Genomics* **71**, 339 (2001).
28. N. Klugbauer *et al.*, *FEBS Lett.* **470**, 189 (2000).
29. S. Tomita *et al.*, *J. Cell Biol.* **161**, 805 (2003).
30. S. Tomita, M. Fukata, R. A. Nicoll, D. S. Bredt, *Science* **303**, 1508 (2004).
31. Y. Fukata *et al.*, *J. Cell Biol.* **169**, 399 (2005).
32. W. Vandenberghe, R. A. Nicoll, D. S. Bredt, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 485 (2005).
33. T. Nakagawa, Y. Cheng, E. Ramm, M. Sheng, T. Walz, *Nature* **433**, 545 (2005).
34. W. Vandenberghe, R. A. Nicoll, D. S. Bredt, *J. Neurosci.* **25**, 1095 (2005).
35. L. Chen, A. El-Husseini, S. Tomita, D. S. Bredt, R. A. Nicoll, *Mol. Pharmacol.* **64**, 703 (2003).
36. S. Tomita *et al.*, *Nature* **435**, 1052 (2005).
37. A. Priel *et al.*, *J. Neurosci.* **25**, 2682 (2005).
38. E. Schnell *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13902 (2002).
39. A. E. El-Husseini, E. Schnell, D. M. Chetkovich, R. A. Nicoll, D. S. Bredt, *Science* **290**, 1364 (2000).
40. J. C. Beique, R. Andrade, *J. Physiol.* **546**, 859 (2003).
41. I. Ehrlich, R. Malinow, *J. Neurosci.* **24**, 916 (2004).
42. M. Yamazaki *et al.*, *Neurosci. Res.* **50**, 369 (2004).
43. D. Turetsky, E. Garringer, D. K. Patneau, *J. Neurosci.* **25**, 7438 (2005).
44. N. Armstrong, E. Gouaux, *Neuron* **28**, 165 (2000).
45. R. Jin, T. G. Banke, M. L. Mayer, S. F. Traynelis, E. Gouaux, *Nat. Neurosci.* **6**, 803 (2003).
46. G. T. Swanson, S. K. Kamboj, S. G. Cull-Candy, *J. Neurosci.* **17**, 58 (1997).
47. D. J. Wyllie, S. F. Traynelis, S. G. Cull-Candy, *J. Physiol.* **463**, 193 (1993).
48. T. C. Smith, L. Y. Wang, J. R. Howe, *J. Neurosci.* **20**, 2073 (2000).
49. N. Rouach *et al.*, *Nat. Neurosci.* **8**, 1525 (2005).
50. D. Zamanillo *et al.*, *Science* **284**, 1805 (1999).
51. S. Tomita, V. Stein, T. J. Stocker, R. A. Nicoll, D. S. Bredt, *Neuron* **45**, 269 (2005).
52. Y. Zheng, J. E. Mellem, P. J. Brockie, D. M. Madsen, A. V. Maricq, *Nature* **427**, 451 (2004).
53. K. Toth, C. J. McBain, *Nat. Neurosci.* **1**, 572 (1998).
54. Y. Lin, V. A. Skeberdis, A. Francesconi, M. V. Bennett, R. S. Zukin, *J. Neurosci.* **24**, 10138 (2004).
55. D. Bowie, E. P. Garcia, J. Marshall, S. F. Traynelis, G. D. Lange, *J. Physiol.* **547**, 373 (2003).

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Cellular Senescence in Aging Primates

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The aging of organisms is characterized by a gradual functional decline of all organ systems. Mammalian somatic cells in culture display a limited proliferative life span, at the end of which they undergo an irreversible cell cycle arrest known as replicative senescence. Whether cellular senescence contributes to organismal aging has been controversial. To reinvestigate this question, we assayed the skin of aging baboons for telomere dysfunction, a recently discovered biomarker of cellular senescence (*1*).

Like humans, baboons have a relatively long life span and show age-dependent telomere shortening (*2*). Baboon skin fibroblasts undergo replicative senescence upon serial passage in culture, with characteristics identical to those of human fibroblasts. Senescent baboon fibroblasts display DNA damage foci that contain phosphorylated histone H2AX (γ-H2AX), activated ataxia-telangiectasia mutated (ATM) kinase ATM(Ser^{1981}), and p53 binding protein (53BP1), and they express activated p53(Ser^{15}) and elevated levels of $p21^{CIP1}$. More than 80% of senescent baboon fibroblasts in vitro display telomere dysfunction–induced foci (TIFs), as determined by the colocalization of γ-H2AX with telomeres.

Full-thickness skin biopsies were obtained from 30 baboons (15 male and 15 female), ranging in age from 5 to 30 years, that were born and raised at the Southwest Regional Primate Center under controlled conditions. The tissue was harvested from the medial aspect of the forearm, a surface that is relatively protected from radiation and injury. The number of dermal fibroblast nuclei containing foci of 53BP1, a marker of DNA double-strand breakage (DSB), increased exponentially with age and reached a value of 30 to 35% in very old (25 to 30 years old) animals (Fig. 1A and fig. S1A). As was found in cultured fibroblasts, 100% of 53BP1 foci colocalized with foci formed by γ-H2AX, another marker of DSB. The majority of 53BP1 foci (62 ± 7.7%) colocalized with telomeric DNA, thus classifying them as TIFs (fig. S1B) (*1*). The frequency of TIF-positive nuclei increased exponentially with age, reaching a value of 15 to 20% in very old animals (Fig. 1B). Dysfunctional telomeres in dermal fibroblasts activate the ATM kinase, as evidenced by the colocalization of γ-H2AX and ATM(Ser^{1981}) (fig. S1C). Ninety-five percent of DNA damage–positive cells displayed staining for four distinct markers of heterochromatin: HP1-β, HIRA (fig. S1, D and E), and histone H3 dimethylated or trimethylated on Lys^9. These data indicate that telomere dysfunction in dermal fibroblasts of baboons activates the ATM signaling pathway and leads to extensive formation of heterochromatin, a hallmark of cellular senescence (*3*). Finally, we observed a high correlation between the presence of TIFs and up-regulation of $p16^{INK4A}$ (fig. S1, F and G). Preliminary analyses indicate that these age-dependent changes are not confined to dermal fibroblasts.

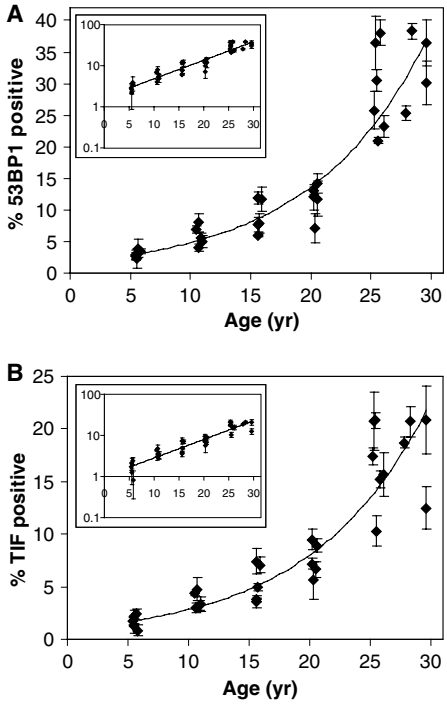

Fig. 1. Telomere dysfunction in skin fibroblasts of living baboons increases with donor age. Skin biopsies were obtained, processed, and imaged, and dermal fibroblasts were scored for the presence of (**A**) 53BP1 foci and (**B**) TIFs as described in (*7*). Thirty individuals in six age groups (five animals per group) were analyzed. Each point represents one animal; there is considerable overlap between the data points, especially at younger ages. 200 to 600 fibroblasts were scored for each animal (error bars show SD). Exponential regressions gave the best fit when applied to the data points [exponential: (A) $R^2 = 0.9136$; (B) $R^2 = 0.8849$; linear: (A) $R^2 = 0.7856$; (B) $R^2 = 0.7884$]. The insets (upper left in each panel) show the same data points plotted on a semi-log scale to further illustrate the exponential accumulation of 53BP1 foci and TIFs with age.

Although replicative senescence of cultured cells has been known for some time, its physiological relevance and contribution to organismal aging have been questioned. This skepticism is largely because of the sparse staining of aged tissues with the senescence-associated β-galactosidase biomarker (*4*). Confidence in this marker has been eroded by findings that its expression can be induced in immortalized cells and even reversed under some conditions (*4*). By using three recently discovered biomarkers (telomere dysfunction, activation of the ATM DNA-damage response, and heterochromatinization of the nuclear genome), we have provided evidence that senescent cells exist in vivo and can account for >15% of the cell population in aged animals. Heterochromatinization is triggered by both replicative and oncogene-induced senescence, is believed to be irreversible, and is associated with profound changes in gene expression (*3*). Thus, the presence of senescent cells in intact tissues at such high frequencies may have profound physiological consequences.

Although we have found a clear in vivo association between telomere dysfunction and senescence, the telomeric DNA damage may not be exclusively due to replicative exhaustion. Oxidative stress increases the rate of telomere attrition, and telomere dysfunction may be triggered by effects other than overt telomere shortening (*5*, *6*). Given the known age-dependent accumulation of oxidative damage in many tissues, it may turn out that telomeres can function in vivo both as replicative and chronological clocks.

References and Notes

1. U. Herbig, W. A. Jobling, B. P. Chen, D. J. Chen, J. M. Sedivy, *Mol. Cell* **14**, 501 (2004).
2. G. M. Baerlocher, J. Mak, A. Roth, K. S. Rice, P. M. Lansdorp, *J. Leukoc. Biol.* **73**, 289 (2003).
3. R. Zhang *et al.*, *Dev. Cell* **8**, 19 (2005).
4. V. J. Cristofalo, *Exp. Gerontol.* **40**, 836 (2005).
5. T. von Zglinicki, *Trends Biochem. Sci.* **27**, 339 (2002).
6. E. H. Blackburn, *FEBS Lett.* **579**, 859 (2005).
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www.sciencemag.org/cgi/content/full/1122446/DC1
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Fig. S1
References

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Architecture of Mammalian Fatty Acid Synthase at 4.5 Å Resolution

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The homodimeric mammalian fatty acid synthase is one of the most complex cellular multienzymes, in that each 270-kilodalton polypeptide chain carries all seven functional domains required for fatty acid synthesis. We have calculated a 4.5 angstrom–resolution x-ray crystallographic map of porcine fatty acid synthase, highly homologous to the human multienzyme, and placed homologous template structures of all individual catalytic domains responsible for the cyclic elongation of fatty acid chains into the electron density. The positioning of domains reveals the complex architecture of the multienzyme forming an intertwined dimer with two lateral semicircular reaction chambers, each containing a full set of catalytic domains required for fatty acid elongation. Large distances between active sites and conformational differences between the reaction chambers demonstrate that mobility of the acyl carrier protein and general flexibility of the multienzyme must accompany handover of the reaction intermediates during the reaction cycle.

Fatty acids are central building blocks of life. They are constituents of biological membranes, energy storage compounds, and messenger substances, and they act as post-translational protein modifiers and modulate gene expression. Consequently, the de novo synthesis of fatty acids is essential for all organisms. It involves a conserved set of chemical reactions for the cyclic stepwise elongation of activated precursors by two-carbon units (*1*, *2*) (Fig. 1). The growing fatty acid is attached to a carrier protein, acyl carrier protein (ACP), throughout its synthesis and is, in mammals, released by a thioesterase (TE) once it reaches 16 or 18 carbon atoms in length (*3*). Although all organisms use variations of this common synthetic scheme, surprisingly, three distinct architectures for fatty acid synthesis have evolved. In bacteria, all reactions are carried out by individual, monofunctional proteins in a dissociated or type II fatty acid synthase (FAS) system (*1*). In contrast, the eukaryotic type I FAS consists of large, multifunctional polypeptides. Fungal FAS is a 2.6-MD $\alpha_6\beta_6$ dodecamer, in which the catalytic domains are distributed over two distinct subunits (*4*, *5*). The FAS of vertebrates and mammals is an α_2 homodimer of a single 270-kD polypeptide. It harbors all catalytic activities required for the synthetic cycle and, in addition, ACP (Fig. 1), making it one of the most complex mammalian enzymes (*2*).

Because of its role in fatty acid synthesis, human FAS is a target for drug development against obesity and obesity-related diseases, including diabetes and cardiovascular disorders. FAS inhibitors have shown potential for weight reduction in animal models (*6*, *7*), though their exact mode of action is under discussion (*8*). FAS is overexpressed in many forms of cancer (*9*), and FAS inhibitors have demonstrated antitumor activity (*10*).

Mammalian FAS serves as a paradigm for a class of multifunctional enzymes known as megasynthases. Members of this family use iterative condensations of carboxylic acid (polyketide synthases, PKS) or amino acid (nonribosomal peptide synthetases, NRPS) building blocks to assemble a variety of secondary metabolites with important biological properties, including immunosuppressants and antibiotics (*11*). Whereas the NRPS are only conceptually related to FAS, modular PKS systems share a common set of catalytic domains with mammalian FAS. Furthermore, the functional domains in mammalian FAS and modular PKS are often arranged in similar order at the sequence level, as exemplified by desoxyerythronolide B synthase (DEBS) (*12*), which is involved in erythromycin biosynthesis.

Currently, no experimental structural information beyond low-resolution electron microscopic reconstructions (*13*, *14*) is available for complete eukaryotic type I FAS or intact PKS modules. However, high-resolution structures of isolated bacterial FAS enzymes yielded important insights into the general reaction mechanisms of fatty acid synthesis (*1*), and crystal structures of recombinant isolated FAS and PKS domains, such as FAS TE (*3*), revealed details about individual active sites of these systems. Here, we present the crystal structure of mammalian FAS at 4.5 Å resolution. It enables accurate placement of all catalytic domains of the fatty acid elongation cycle and provides insight into domain organization in mammalian FAS. ACP and TE domains could not be placed, presumably because of their inherent flexibility.

Structure determination. FAS was purified from porcine mammary gland by established procedures (*15*). On the basis of amino acid sequence identities between human, bovine or rat, and porcine FAS of more than 70%, the latter is representative for all mammalian FAS systems. FAS crystals in the monoclinic space group $P2_1$ with a maximum size of 0.40 mm by 0.07 mm by 0.02 mm were grown by the vapor-diffusion method using polyethylene glycol 3350 as the precipitant at pH 6.7 to 7.3 and diffracted to a maximum resolution of 4.3 Å. Experimental phases to 4.5 Å resolution were determined using multiple isomorphous replacement with anomalous scattering and improved by density modification. Secondary-structure elements are clearly recognizable in most parts of the molecule, as expected for a 4.5 Å–resolution crystallographic map. Based on the identification of secondary-structure elements, all catalytic domains of the fatty acid

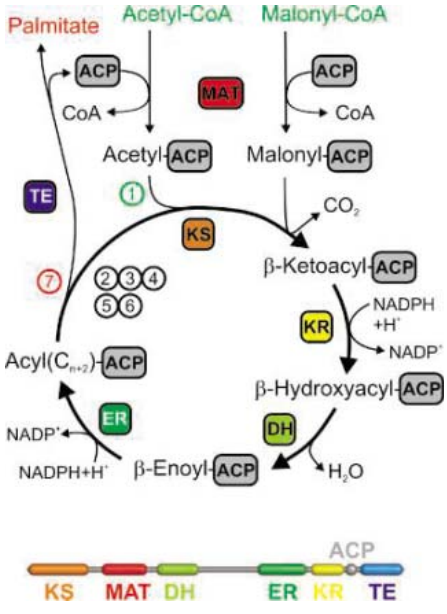

Fig. 1. Catalytic cycle and domain organization. The reaction cycle of FAS is initiated by the transfer of the acyl moiety of the starter substrate acetyl-CoA to the acyl carrier protein (ACP, gray) catalyzed by the malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT, red), which also transacylates the malonyl group of the elongation substrate malonyl-CoA to ACP. The β-ketoacyl synthase (KS, orange) catalyzes the decarboxylative condensation of the acyl intermediate with malonyl-ACP to a β-ketoacyl-ACP intermediate, acetoacetyl-ACP in the first cycle. The β-carbon is processed by nicotinamide adenine dinucleotide phosphate (NADPH)–dependent reduction through β-ketoacyl reductase (KR, yellow). The resulting β-hydroxyacyl-ACP is dehydrated by a dehydratase (DH, light green) to a β-enoyl intermediate, which is reduced by the NADPH-dependent β-enoyl reductase (ER, dark green) to yield a four-carbon acyl substrate for further cyclic elongation with two-carbon units derived from malonyl-CoA until a substrate length of C_{16} to C_{18} is reached. Finally, the product is released from the ACP by the thioesterase (TE, blue). The lower panel shows the linear domain organization of mammalian FAS.

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elongation cycle were placed into the electron density map (Fig. 2). However, it was not possible to unambiguously trace the interdomain linking regions. The ACP and TE domains could not be placed with confidence most likely because of their inherent flexibility or flexible attachment and have not been included in the current model.

Overall structure and domain assignment. Mammalian FAS adopts an X-shape with a central body extended at the upper and lower ends by "arms" and "legs," respectively. The overall dimensions of the complex of 210 Å by 180 Å by 90 Å are in good agreement with earlier low-resolution electron microscopic observations (*13*, *14*, *16*) (Fig. 3). An approximate two-fold rotational axis of symmetry relating the two monomers of homodimeric FAS extends vertically through the FAS body, as indicated in Fig. 3. However, an assignment of domains to the two distinct monomers is not yet possible, because the current resolution is insufficient to unambiguously trace interdomain connecting regions.

Even though the sequence identity between individual proteins of the bacterial type II FAS system and mammalian FAS is low in some areas, most of the mammalian FAS domains adopt a fold similar to that of their bacterial counterparts (Table 1 and Fig. 2). Starting from the N terminus of mammalian FAS, the β-ketoacyl synthase (KS) domains are located in the lower body (Fig. 3, A and C) and closely resemble the *Escherichia coli* KS I (FabB) (*17*) (Fig. 2A). The malonyl-coenzyme A (CoA)-/acetyl-CoA-ACP-transacylase (MAT) domains form the two "legs" of FAS (Fig. 3, A and C) and are homologs of the bacterial malonyl transferase (FabD) (*18*) (Fig. 2B). The dehydratase (DH) domains comprise the upper body of FAS (Fig. 3A). Despite a lack of sequence homology, each of these domains adopts a "double hot dog" fold (Fig. 2C) closely related to the fold of the dimeric bacterial dehydratases FabA (*19*) and FabZ (*20*) and related pseudo-dimeric eukaryotic enzymes (*21*). The β-enoyl reductase (ER) domain is a member of the medium-chain dehydrogenase family (*22*). The best structural match was obtained with a zinc-free bacterial quinone reductase (Fig. 2D) (*23*) with the application of a small rotation of the catalytic relative to the nucleotide-binding domain. Notably, the structure of a PKS ER domain fragment [Protein Data Bank (PDB) accession code: 1pqw] closely resembles that of the cofactor-binding domain. The ER domains sit on top of the DH domains at the upper end of the FAS body (Fig. 3, A and B). The last catalytic domains of the fatty acid elongation cycle, the β-ketoacylreductase (KR) domains, are located adjacent to the ER domains in the FAS arms (Fig. 3, A and B). KR belongs to the short-chain dehydrogenase family (*24*), comprising bacterial enoyl- and ketoreductases, and was modeled with *E. coli* KR (FabG) (*25*) (Fig. 2E).

At the end of one arm (right in Fig. 3, A and B), a blurred volume of electron density is observed, which is nearly completely absent at the end of the other arm. Most likely, it represents a particularly mobile part of FAS, which is only partly stabilized by the observed crystal contacts. It might be interpreted as arising from the C-terminal ACP and TE domains of one monomer based on the size of the density and the close vicinity to the KR domain, which is directly preceding ACP and TE in linear sequence. This assignment agrees with the location of the TE domain at the ends of the long axis of FAS inferred from visualization of antibody complexes of harderian gland FAS (*16*) and the approximately equidistant location of a labeled ACP phosphopantheteine to both types of reductase centers (*26*). The high inherent flexibility of the TE domain has already been demonstrated by limited proteolysis (*27*), fluorescence and mutational studies (*28*), and the functional interaction of FAS with thioesterase II (*29*). Furthermore, structures of the isolated human TE (*3*) and rat ACP domains (*30*) suggest the presence of considerable intradomain flexibility.

The central ~650 residues of mammalian FAS have previously been assigned as the "core" or "interdomain" (*31*) (Fig. 1), which is characterized by the absence of catalytic centers and lower sequence conservation and has been implicated in FAS dimerization (*32*). The structure of mammalian FAS reveals that the DH domain that precedes the "core" forms a "double hot dog" fold and has about twice the expected size at sequence level (*2*). Conse-

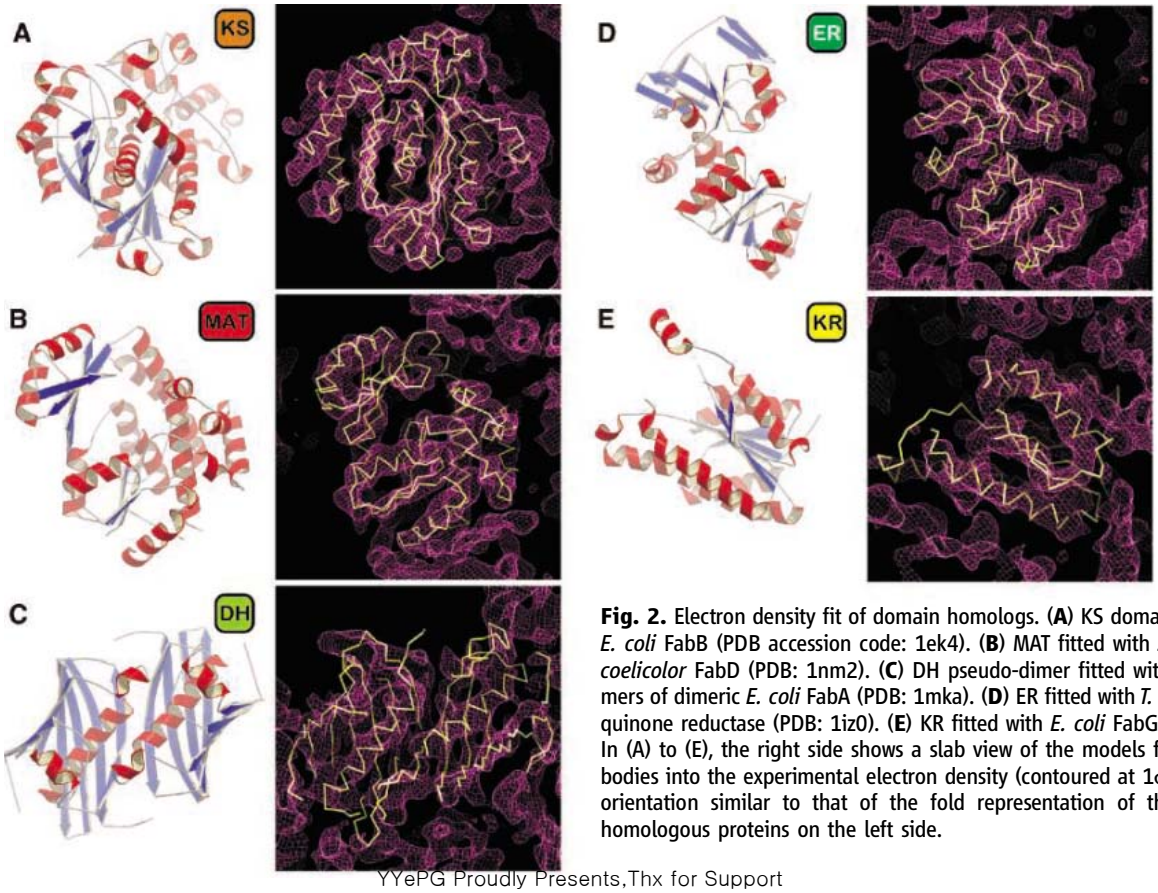

Fig. 2. Electron density fit of domain homologs. (**A**) KS domain fitted with *E. coli* FabB (PDB accession code: 1ek4). (**B**) MAT fitted with *Streptomyces coelicolor* FabD (PDB: 1nm2). (**C**) DH pseudo-dimer fitted with two monomers of dimeric *E. coli* FabA (PDB: 1mka). (**D**) ER fitted with *T. thermophilus* quinone reductase (PDB: 1iz0). (**E**) KR fitted with *E. coli* FabG (PDB: 1i01). In (A) to (E), the right side shows a slab view of the models fitted as rigid bodies into the experimental electron density (contoured at 1σ level) in an orientation similar to that of the fold representation of the respective homologous proteins on the left side.

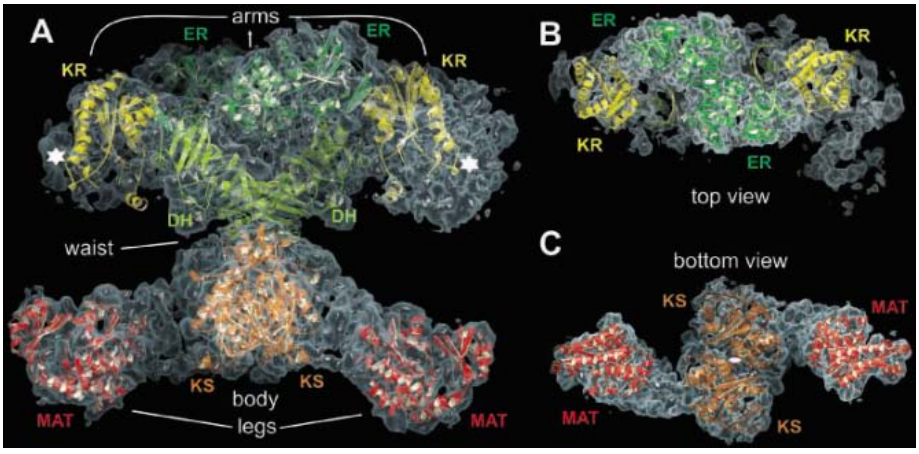

Fig. 3. Structural overview. Fitted domains (colored as in Fig. 1) are shown with a semitransparent surface representation of the experimental electron density (contoured at 1σ level) around one dimeric FAS. White stars indicate the pseudosymmetry-related suggested attachment regions for ACP and TE, where only on the right side a large volume of blurred density is visible. (**A**) Front view: FAS consists of a lower part comprising the KS (lower body) and MAT domains (legs) connected at the waist with an upper part formed by the DH, ER (upper body), and KR domains (arms). (**B**) Top view of FAS with the ER and KR domains resting on the DH domains. (**C**) Bottom view showing the arrangement of the KS and MAT domains and the continuous electron density between the KS and MAT domains. In (A) to (C), the approximate position of the pseudo-twofold dimer axis is indicated by an arrow and ellipsoid.

Table 1. Domains of the mammalian FAS elongation cycle, and their structural homologs and functional analogs.

Mammalian FAS domain	Oligomerization state	Placed structural homologs	Oligomerization state	Functionally related bacterial FAS proteins
KS	Dimeric	FabB, *E. coli*	Dimeric	FabB, FabF, FabH
MAT	Monomeric	FabD, *S. coelicolor*	Monomeric	FabD
DH	Pseudo-dimeric*	FabA, *E. coli*	Dimeric	FabA, FabZ
ER	Dimeric	Quinone reductase, *T. thermophilus*	Dimeric	FabI, FabK, FabL
KR	Monomeric	FabG, *E. coli*	Tetrameric	FabG

*The DH pseudo-dimer occurs within one polypeptide chain of FAS and is not formed across the dimer interface between the two FAS subunits. The full FAS dimer thus contains two individual pseudo-dimeric DH domains.

quently, the length of the catalytically inactive ''core'' is reduced to about 450 residues. However, no additional electron density corresponding to a compact domain of such size could be identified, suggesting that it may be disordered or distributed in between other domains serving a structural role (fig. S1, A and B).

Intersubunit and interdomain connections. In the early, classical model, mammalian FAS was represented as an H-shaped dimer with linear head-to-tail arrangement of subunits, which are centrally connected by the noncatalytic ''core'' (*32*). On the basis of structural and functional characterization of recombinant mutant FAS and complementation assays, Smith and co-workers revised the initial model and depicted FAS as an intertwined head-to-head dimer with distinct conformations at various stages of its catalytic cycle (*2*, *14*). The current structure fundamentally agrees with the revised model and demonstrates that mammalian FAS is, indeed, an intertwined dimer with a large dimerization interface running through the body of the molecule, perpendicular to the interface proposed in the classical scheme (*13*).

The KS domains dimerize in the same way as the homologous homodimeric *E. coli* KS I enzyme (FabB) (*17*) (fig. S1D) with their N termini in close proximity; this is in agreement with cross-links between the N termini of companion KS domains via engineered cysteine residues (*33*). Another important contribution to the dimer interface comes from the ER domain. Based on the placement of the homologous *Thermus thermophilus* quinone oxidoreductase monomers into the electron density, also the ER domains of mammalian FAS associate in the same way as the isolated homologous bacterial enzyme (*23*). The interaction is guided by the formation of a continuous 12-stranded β sheet between the nucleotide-binding domains of the monomers (fig. S1C). Consistent with a role of the ER domain in dimerization, the thermal stability of homodimeric ER active-site mutants of FAS is substantially reduced (*34*). As estimated from the structures of the homologous bacterial enzymes, the two homophilic interactions between the ER and KS domains contribute ~5000 Å^2 to the total dimer interface of mammalian FAS. Substantial intersubunit contacts are also formed along the pseudo-twofold axis in the waist region by the lower parts of the DH domains (Fig. 3A). The unassigned interspersed regions and interdomain connections could mediate further intersubunit interactions (fig. S1).

In the current structural model, the KS domains are surrounded by linking regions interconnecting KS/MAT and MAT/DH, which apparently build up a mixed α/β-fold adapter between KS and MAT. At their top, the KS domains are contacting the lower part of the DH domains, connecting the lower and upper part of the body in the waist region. The spatial arrangement of these domains may explain why the shortest recombinant N-terminal FAS construct with KS activity must, in addition to KS, also enclose MAT and part of the DH domain, which are surrounding KS in the current structure, and why this construct shows dimerization properties similar to those of the full-length FAS (*33*). The example of KR demonstrates that the oligomerization contacts are not transferred from the isolated bacterial homologs to the mammalian FAS domains as a rule (Table 1): Whereas the *E. coli* KR (FabG) is tetrameric (*25*), the two KR domains of mammalian FAS do not interact.

Active sites and reaction chambers. The placement of homologous structures with known catalytic mechanism into the 4.5 Å electron density map accurately defines the positions of active sites of mammalian FAS. During the catalytic cycle of FAS, the growing acyl chain remains attached to the phosphopantetheine arm of ACP, with the exception of the temporal transfer to the KS active site. From the location of the KR domain, to which the ACP is tethered by only a short linker, and the position of the TE domain inferred from earlier work (*16*), it is possible to establish the approximate position of ACP close to the ends of the FAS ''arms.'' On the basis of the structural information presented here, the active centers of FAS fall into two groups, according to their accessibility to one or the other ACP domain: one complete set of domains required for productive elongation in each of the two lateral clefts (Fig. 4A). In the observed conformation of FAS, it appears difficult for the ACP to reach any of the active centers of the opposite side cleft—not only because of long distances, but also because the KS and DH domains protrude sideways and block the way around the body to the other cleft (Fig. 4B). Consequently, the lateral clefts define two preferred reaction chambers (Fig. 4A).

In the order of the FAS reaction cycle, the lower part of the reaction chamber comprises the first two enzymatic domains, with MAT forming the legs and KS the central body. The distance between their deep-set active sites is ~71 Å;

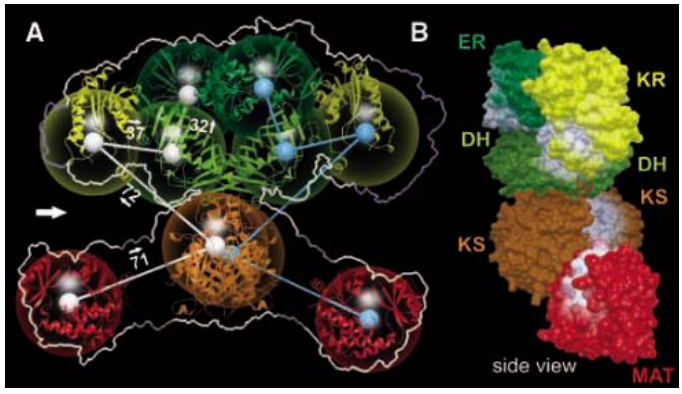

Fig. 4. Active sites and reaction chamber. (**A**) Front view of FAS with ribbon representations of fitted domains colored as in Fig. 1. The overall shape is indicated by the outline of electron density; gray and blue colors of the outline mark the nonmodeled KS/MAT interconnection and suggested ACP/TE location, respectively. The positions of active sites in the two reaction chambers are indicated by solid white and blue spheres. Hollow spheres in domain colors that surround the active sites denote the length of the phosphopantheteine arm, reflecting how close ACP has to approach the individual domains during the catalytic cycle. The active sites are connected in order of the reaction sequence with distances between the active sites indicated for the left reaction chamber. (**B**) Side view into one reaction chamber as indicated by a white arrow in (A); for clarity, only surface representations of the fitted domains are shown. Active sites of fitted domains of one reaction chamber are indicated by a color gradient to white on the respective surfaces.

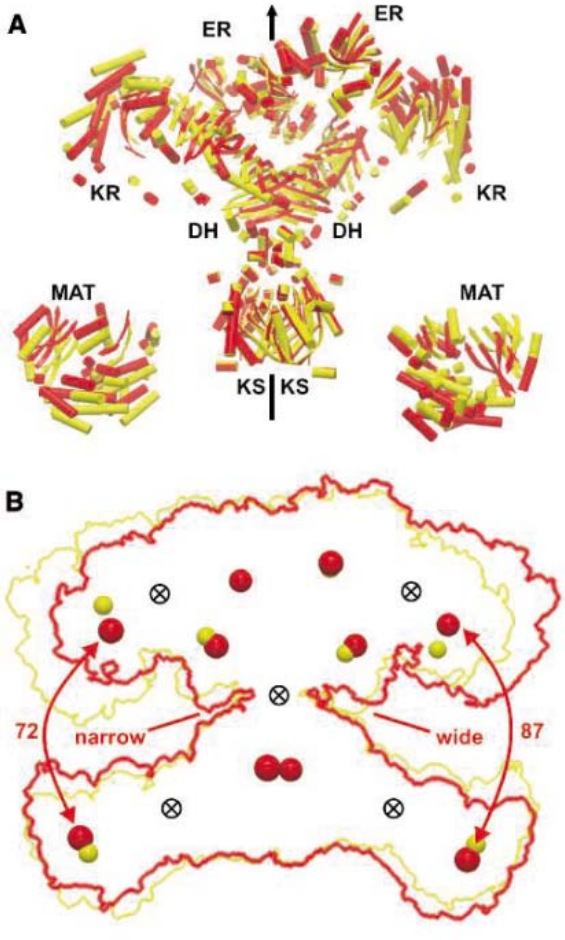

Fig. 5. Interdomain hinges and conformational variability. For structural comparison, the FAS dimer is superimposed onto itself by applying the transformation relating the dimer of KS domains as indicated by an arrow. As a result, the left reaction chamber is transformed onto the right one and vice versa. The original orientation is shown in red, the transformed one in yellow. (**A**) Only secondary structural elements of the fitted domains are shown. Largest differences are observed for the positions of the KR and MAT domains at the periphery. The approximate position of the pseudo-twofold dimer axis is indicated by an arrow. (**B**) Experimental electron density is schematically shown as an outline. The positions of active sites are indicated by spheres, hinges by crossed circles. The left reaction chamber is considerably narrower than the right one with a difference of distances between the KR and MAT active sites of about 15 Å as indicated for the original orientation in red.

distances between the entrances to the substrate-binding clefts are considerably shorter. The visible connections in electron density between the KS and MAT domains suggest that the connected domains belong to one monomer (Fig. 3C). Based on this assumption, the KS and MAT domains of each reaction chamber are contributed by different monomers. The upper part of the chamber is composed of the three β-carbon processing domains: KR, DH, and ER. The active site of KR is located at a distance of ~72 Å to the preceding domain in the reaction sequence, KS. The DH active site resides only about 37 Å away from the KR active site, but its substrate-binding cleft points in a slightly different direction. The ER and DH domains are in close proximity, with a distance of ~32 Å between their active centers. The arrangement of these two catalytic sites would even allow ACP to shuttle the substrates between them without substantially changing its position.

Conformational variability and reaction mechanism. Considering the overall shape of FAS, crystallized in the absence of cofactors or substrates, it is noticeable that its conformation results in two nonidentical reaction chambers. One chamber (Fig. 3A, left; Fig. 5, A and B) is considerably narrower than the other: The distance between the active centers of the peripheral MAT and KR domains is 72 Å in the narrow chamber, but 87 Å in the wide chamber (Fig. 5B). Surprisingly, also electron microscopic reconstructions of mammalian FAS in the presence of substrates (*13*, *14*) frequently yielded asymmetric structures. Together, these observations might suggest a physiological relevance of the observed asymmetry of FAS, although we cannot exclude that the crystallized FAS was only trapped in one out of multiple possible conformations. A superposition of FAS onto itself based on the twofold relation between the central KS domains reveals hinge regions that cause the observed asymmetry of the clefts (Fig. 5B). The central hinge is located very close to the substrate-binding lids of the KS domain dimer in the "waist" region connecting the lower and upper parts of FAS. Notably, the crystal structure of the homodimeric bacterial homolog of KS, FabB, revealed an asymmetric mode of substrate binding (*17*). Furthermore, even under saturating substrate conditions, the KS domain of FAS binds single substrates only substoichiometrically (*35*). Therefore, it is tempting to speculate that asymmetric binding and release of substrates by the KS dimer may affect the conformations of the KS substrate-binding loops at the waist region of the FAS and induce opening and closing of the reaction chambers.

Around a second hinge, the MAT domains in the "legs" of FAS undergo a slight up-and-down motion relative to the KS domain (Fig. 5B). The extended interface on both sides of the MAT/KS joint (Fig. 3A), however, appears to preclude large-scale motions of the MAT domain. A third hinge resides at the less solid contact between the KR domains and the pairs of ER and DH domains, which are held together by a substantial interface (Fig. 5B). The phosphopantetheine group of ACP obviously does not serve as a "swinging arm," as proposed in very early models of type I FAS (*36*). As indicated in Fig. 4A, its length is just sufficient to reach the deep-set active centers, even assuming that the ACP is in close proximity to the respective domains. In the dissociated bacterial system, substrate-loaded ACP interacts transiently with individual FAS proteins through a proposed common ACP-binding motif in these proteins (*37*). On the basis of the observed structural homology, such guiding in-

teractions might also facilitate the entry of ACP-bound substrates into deep-set active sites in mammalian FAS.

From the arrangement of active sites within one chamber, it is obvious that considerable flexibility of ACP, which might result from a combination of its internal and linker flexibility, combined with modest domain motions, as indicated by the observed asymmetry, are required to enable access of ACP to all domains of one cleft, likely involving KS and MAT domains from different FAS subunits. However, biochemical studies have established the existence of redundant alternative routes for fatty acid elongation by FAS (*34*, *38*), which increase the overall efficiency of FAS (*2*). The interaction between the ACP-bound substrate and the DH, ER, KR, and TE domains is almost exclusively an intrasubunit process. However, loading and condensation may involve either intersubunit or intrasubunit interactions between KS and MAT with ACP (*39*). On the basis of mutant complementation and cross-linking, 20 to 35% of all elongation cycles proceed via the alternative route, involving intrasubunit interplay between ACP and KS (*2*, *40*). Even a heterodimeric FAS with all catalytic domains of one subunit inactivated by mutation still exhibits 16% of the wild-type activity (*38*). Considering the large distance between the involved domains—for example, between the suggested position of one ACP and both MAT domains—the presented structure accounts for a major conformation but would not explain a minor alternative synthetic route. The existence of multiple conformations of FAS and their dependence, for example, on the presence of substrates were inferred from early cross-linking experiments (*41*) and have recently been observed in electron microscopic studies (*14*).

Implications for the megasynthase family. Mammalian FAS is a paradigm for the structural organization of modular PKS. These huge homodimeric proteins are assembled from multiple modules, each capable of catalyzing one elongation step, equivalent to a single elongation cycle in FAS, with various extends of β-carbon processing. The minimal PKS module consists of a KS, an acyl transferase (AT), and an ACP domain. Extensions of this minimal set by β-carbon processing domains, together with the substrate preference of the AT domain, determine the product of a particular module. Under physiological conditions, substrate transport through PKS modules is colinear with the arrangement of modules at sequence level, such that the order of distinct modules determines the chemical structure of products, which are released by a terminating thioesterase (*11*). Homodimeric modular PKS have been envisioned as parallel, interwound supramolecular helices with a structural core formed by KS, AT, and ACP domains and off-axis extensions by varying numbers of β-carbon processing domains (*42*). This arrangement is represented in the structure of mammalian FAS by the KS/MAT domain blocks in the lower part of mammalian FAS, dimerized via the homophilic interactions of the KS domain and segregated upper segments comprising the β-carbon processing, ACP, and TE domains.

The peripheral positioning of MAT domains of mammalian FAS in the "leg" region and their attachment through an interface formed by noncatalytic linker regions might indicate that the related AT domains of modular PKS could also be placed off-axis without a role in dimerization. Such positioning agrees well with the existence of modular PKS lacking an internal AT domain, in which the AT functionality is supplied in trans by monofunctional AT proteins (*43*). In mammalian FAS, the downstream domain block beyond MAT, including β-carbon processing domains, ACP, and TE, is in contact with the substrate-binding region of the KS domain in the waist region, and it might be speculated that a similar arrangement in PKS modules could provide the possibility of cross talk between KS and downstream domains. Of course, the structure of cyclically acting mammalian FAS cannot provide direct insight into intermodular substrate shuttling in PKS. However, the domain architecture revealed here provides a first structural view onto the common scaffold of mammalian FAS and modular PKS.

Conclusion. The overall architecture of mammalian FAS has been revealed by x-ray crystallography at intermediate resolution. The dimeric synthase adopts an asymmetric X-shaped conformation with two reaction chambers on each side formed by a full set of enzymatic domains required for fatty acid elongation, which are separated by considerable distances. Substantial flexibility of the reaction chamber must accompany the handover of reaction intermediates during the FAS cycle, and further conformational transitions are required to explain the presence of alternative inter- and intrasubunit synthetic routes in FAS. The results presented here provide a new structural basis to further experiments required for a detailed understanding of the complex mechanism of mammalian FAS. Furthermore, continued work on the current crystal system may ultimately provide an atomic model of mammalian FAS.

References and Notes

1. S. W. White, J. Zheng, Y. M. Zhang, C. O. Rock, *Annu. Rev. Biochem.* **74**, 791 (2005).
2. S. Smith, A. Witkowski, A. K. Joshi, *Prog. Lipid Res.* **42**, 289 (2003).
3. B. Chakravarty, Z. Gu, S. S. Chirala, S. J. Wakil, F. A. Quiocho, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15567 (2004).
4. E. Schweizer, J. Hofmann, *Microbiol. Mol. Biol. Rev.* **68**, 501 (2004).
5. S. Jenni, M. Leibundgut, T. Maier, N. Ban, *Science* **311**, 1263 (2006).
6. T. M. Loftus *et al.*, *Science* **288**, 2379 (2000).
7. M. V. Kumar, T. Shimokawa, T. R. Nagy, M. D. Lane, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 1921 (2002).
8. F. P. Kuhajda, L. E. Landree, G. V. Ronnett, *Trends Pharmacol. Sci.* **26**, 541 (2005).
9. F. P. Kuhajda *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6379 (1994).
10. F. P. Kuhajda *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3450 (2000).
11. J. Staunton, K. J. Weissman, *Nat. Prod. Rep.* **18**, 380 (2001).
12. S. Donadio, M. J. Staver, J. B. McAlpine, S. J. Swanson, L. Katz, *Science* **252**, 675 (1991).
13. J. Brink *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 138 (2002).
14. F. J. Asturias *et al.*, *Nat. Struct. Mol. Biol.* **12**, 225 (2005).
15. Materials and methods are available as supporting material on *Science* Online.
16. T. Kitamoto, M. Nishigai, T. Sasaki, A. Ikai, *J. Mol. Biol.* **203**, 183 (1988).
17. J. G. Olsen, A. Kadziola, P. von Wettstein-Knowles, M. Siggaard-Andersen, S. Larsen, *Structure* **9**, 233 (2001).
18. A. T. Keatinge-Clay *et al.*, *Structure* **11**, 147 (2003).
19. M. Leesong, B. S. Henderson, J. R. Gillig, J. M. Schwab, J. L. Smith, *Structure* **4**, 253 (1996).
20. M. S. Kimber *et al.*, *J. Biol. Chem.* **279**, 52593 (2004).
21. K. M. Koski, A. M. Haapalainen, J. K. Hiltunen, T. Glumoff, *J. Mol. Biol.* **345**, 1157 (2005).
22. E. Nordling, H. Jornvall, B. Persson, *Eur. J. Biochem.* **269**, 4267 (2002).
23. Y. Shimomura, Y. Kakuta, K. Fukuyama, *J. Bacteriol.* **185**, 4211 (2003).
24. B. Persson, Y. Kallberg, U. Oppermann, H. Jornvall, *Chem. Biol. Interact.* **143-144**, 271 (2003).
25. A. C. Price, Y. M. Zhang, C. O. Rock, S. W. White, *Biochemistry* **40**, 12772 (2001).
26. Z. Y. Yuan, G. G. Hammes, *J. Biol. Chem.* **261**, 13643 (1986).
27. J. S. Mattick *et al.*, *J. Biol. Chem.* **258**, 15300 (1983).
28. A. K. Joshi, A. Witkowski, H. A. Berman, L. Zhang, S. Smith, *Biochemistry* **44**, 4100 (2005).
29. L. J. Libertini, S. Smith, *J. Biol. Chem.* **253**, 1393 (1978).
30. M. A. Reed *et al.*, *Org. Biomol. Chem.* **1**, 463 (2003).
31. A. K. Joshi, S. Smith, *J. Biol. Chem.* **268**, 22508 (1993).
32. S. S. Chirala, A. Jayakumar, Z. W. Gu, S. J. Wakil, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3104 (2001).
33. A. Witkowski *et al.*, *Chem. Biol.* **11**, 1667 (2004).
34. V. S. Rangan, A. K. Joshi, S. Smith, *Biochemistry* **40**, 10792 (2001).
35. A. Witkowski, A. K. Joshi, S. Smith, *Biochemistry* **41**, 10877 (2002).
36. F. Lynen, *Biochem. J.* **102**, 381 (1967).
37. Y. M. Zhang, H. Marrakchi, S. W. White, C. O. Rock, *J. Lipid Res.* **44**, 1 (2003).
38. A. K. Joshi, V. S. Rangan, A. Witkowski, S. Smith, *Chem. Biol.* **10**, 169 (2003).
39. V. S. Rangan, A. K. Joshi, S. Smith, *J. Biol. Chem.* **273**, 34949 (1998).
40. A. K. Joshi, A. Witkowski, S. Smith, *Biochemistry* **37**, 2515 (1998).
41. W. X. Tian, R. Y. Hsu, Y. S. Wang, *J. Biol. Chem.* **260**, 11375 (1985).
42. J. Staunton *et al.*, *Nat. Struct. Biol.* **3**, 188 (1996).
43. B. Shen, *Curr. Opin. Chem. Biol.* **7**, 285 (2003).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5765/1258/DC1
Materials and Methods
Fig. S1
Table S1

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Architecture of a Fungal Fatty Acid Synthase at 5 Å Resolution

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All steps of fatty acid synthesis in fungi are catalyzed by the fatty acid synthase, which forms a 2.6-megadalton $\alpha_6\beta_6$ complex. We have determined the molecular architecture of this multienzyme by fitting the structures of homologous enzymes that catalyze the individual steps of the reaction pathway into a 5 angstrom x-ray crystallographic electron density map. The huge assembly contains two separated reaction chambers, each equipped with three sets of active sites separated by distances up to ~130 angstroms, across which acyl carrier protein shuttles substrates during the reaction cycle. Regions of the electron density arising from well-defined structural features outside the catalytic domains separate the two reaction chambers and serve as a matrix in which domains carrying the various active sites are embedded. The structure rationalizes the compartmentalization of fatty acid synthesis, and the spatial arrangement of the active sites has specific implications for our understanding of the reaction cycle mechanism and of the architecture of multienzymes in general.

Fatty acid synthesis in fungi is compartmentalized in large multifunctional enzymes: the fatty acid synthases (FASs). They function as molecular assembly lines and increase the rate of synthesis by channeling substrates between active sites and achieving high local concentrations of intermediates (*1*–*3*). De novo fatty acid synthesis involves the transfer of activated acetyl and malonyl substrates from coenzyme A (CoA) to the prosthetic phosphopantetheine group of acyl carrier protein (ACP) by acetyl transferase (AT) and malonyl/palmitoyl transferase (MPT) (Fig. 1). Ketoacyl synthase (KS) condenses them by malonyl decarboxylation to acetoacetyl-ACP, which is further modified at the β-carbon position by ketoacyl reductase (KR), dehydratase (DH), and enoyl reductase (ER) to yield butyryl-ACP. These reactions are repeated six times, resulting in the sequential incorporation of seven two-carbon units. In fungi, the palmitoyl end product is transferred back from ACP to CoA by MPT, whereas in the mammalian system, the fatty acid is released from ACP by a thioesterase (*4*).

Despite the conserved reaction pathway, the molecular architectures of FAS systems differ considerably: Most bacteria and plants possess a FAS type-II system with dissociated enzymes encoded by separate genes (*5*). Highly integrated FAS type-I multienzyme systems are present in mammals, with all catalytic activities taking place on an α_2 homodimer of 270-kD polypeptides (*4*), and in fungi, where the individual enzymatic functions are distributed on a 210-kD α chain and a 230-kD β chain (Fig. 1) (*3*). In addition, the C terminus of the fungal α chain encodes the phosphopantetheine transferase (PT) required for ACP activation by phosphopantetheine transfer (*6*). Fungal FAS is an $\alpha_6\beta_6$ heterododecameric complex with a molecular weight of 2.6 MD. According to electron microscopic results, FAS assembles into a hollow rotational ellipsoid with 32-point group symmetry (*7*–*12*).

Several *Aspergillus* strains use a closely homologous FAS (HexA/B) to produce hexanoic acid, a precursor substrate for the synthesis of aflatoxin and related mycotoxins (*13*–*15*). A related FAS type-I system is also present in *Mycobacteria* and *Corynebacteria*, encoded by a single large gene with fused β and α chains. It is proposed to synthesize precursors for mycolic acids, which are deposited in the bacterial cell wall and form an effective protective layer (*16*, *17*).

Several x-ray crystallographic and nuclear magnetic resonance structures of homologous FAS type-II proteins in complex with cofactors, substrates, or inhibitors illustrate the molecular basis of individual chemical reactions at the atomic level. However, it is not understood how multiple catalytic domains assemble into large multifunctional complexes and form a reaction environment suitable for the coordinated biosynthesis of fatty acids. To understand the architecture of fungal FAS, we calculated a 5 Å–resolution x-ray crystallographic electron density map, into which we positioned homologous crystal structures for the enzymes KS, KR, DH, MPT, and AT, together with a triose phosphate isomerase (TIM) barrel that constitutes the flavin mononucleotide (FMN)–binding domain of the ER. The accurate positions and orientations of the catalytic domains and their active sites define the architecture of the assembly with implications for the understanding of its function.

Structure determination. FAS was isolated from the filamentous fungus *Thermomyces lanuginosus* and crystallized. Initial phases were obtained at 8 Å resolution from crystals derivatized with heavy atom clusters (*18*). Phases were then extended by density modification to approximately 5 Å resolution, as judged from the appearance of α helices and β sheets in the resulting experimental electron density map. Although, at this resolution, the protein backbone cannot be unambiguously traced, distinct protein folds formed by combinations of various secondary structure elements are readily recognizable. This allowed us to interpret the map by placing homologous high-resolution structures of individual domains into the electron density map.

Identification of functional domains. Bacterial KS adopts a thiolase fold and forms homodimers in solution and in several x-ray structures (*19*). Consequently, we inspected the FAS electron density map along the twofold-symmetry axes and could identify the thiolase fold. The structure of the bacterial KS homolog fits almost perfectly into the density (Fig. 2A), particularly in the proximity of the catalytic center. Within the FAS complex, KS forms three dimers and preserves the dimer interface of its bacterial counterpart.

In the vicinity of the twofold axis on the other side of the complex, a characteristic four-helix bundle was recognized as one of the dimerization interfaces in the type-II tetrameric KR homolog of *Brassica napus* (*20*). KR is a nicotinamide adenine dinucleotide phosphate (NADPH)–dependent enzyme containing a dinucleotide-binding Rossmann fold. The KR domains fit remarkably well into our electron density map (Fig. 2B) and form dimers that correspond to one of the dimers observed in the dissociative type-II FAS system.

The closest sequence homolog with known structure to fungal DH is the human peroxisomal 2-enoyl-CoA hydratase 2 involved in β oxidation of fatty acids (*21*). It is a pseudo-dimer, with two subsequent "hot dog" folds, which together form a large characteristic β sheet. This feature and the surrounding electron

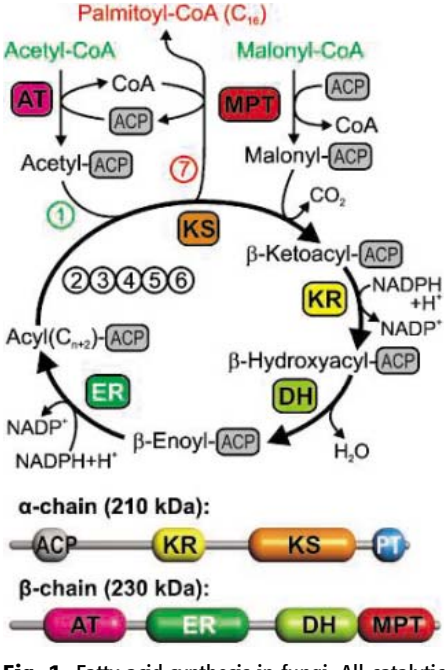

Fig. 1. Fatty acid synthesis in fungi. All catalytic domains necessary for the synthesis of C_{16} fatty acids are distributed on two multidomain polypeptide chains.

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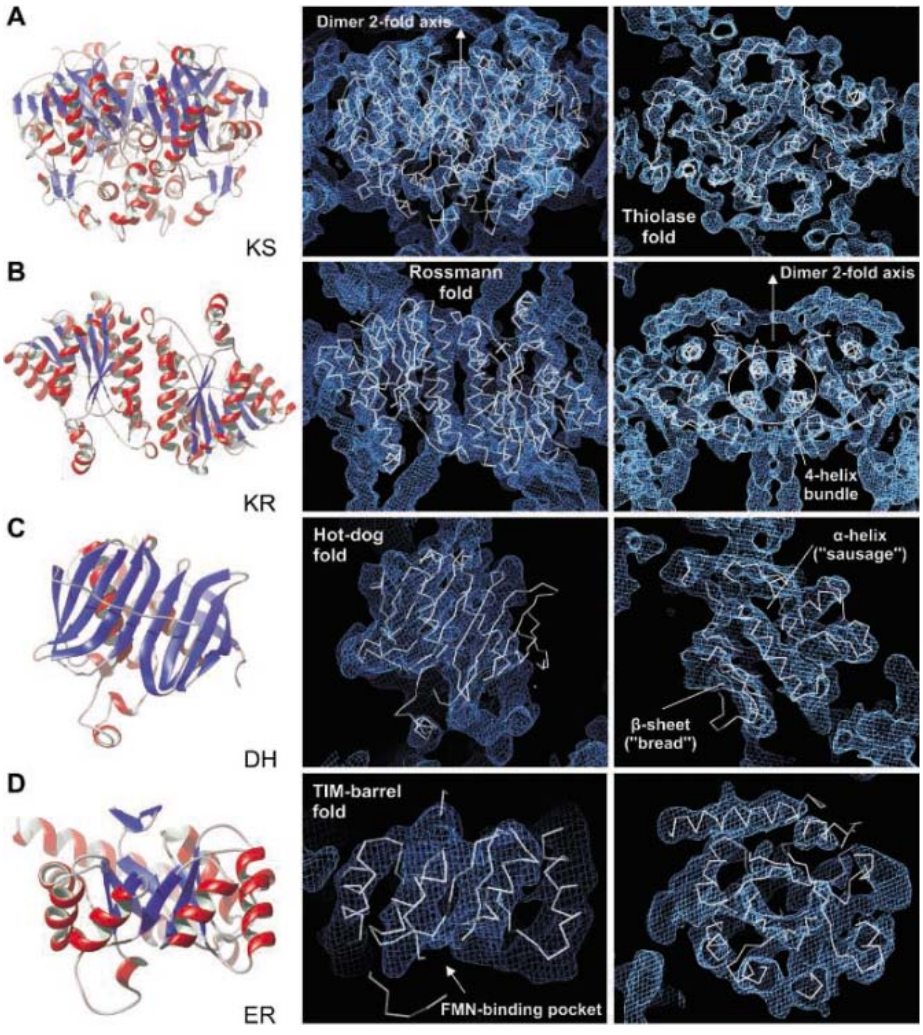

Fig. 2. Fitting of homologous catalytic domains (left column) into the 5 Å electron density map contoured at 1.2σ (middle and right columns). (**A**) KS dimer [Protein Data Bank (PDB) accession code 1DD8] fitted into the electron density along the twofold noncrystallographic symmetry axis of the FAS assembly. (**B**) KR dimer (PDB accession code 1EDO) fitted into the electron density map. KR dimerizes via a four-helix bundle. (**C**) The DH contains a characteristic extended β sheet and adopts a fold closely related to the pseudo-dimeric structure observed in a hydratase of the β-oxidation pathway (PDB accession code 1S9C). (**D**) Side and top views of the TIM-barrel fold, which represents the FMN-binding part of the fungal ER. The closest fit to the electron density map was obtained using a FMN-containing oxidoreductase (PDB accession code 1GOX).

density allowed the fit of both the C-terminal half, which shows highest sequence homology to fungal FAS and harbors the catalytic site of the hydratase, and the N-terminal half, which is involved in the binding of bulky substrates (Fig. 2C). DH structures of the bacterial FAS homologs (*22*, *23*) resemble the peroxisomal hydratase fold but are true homodimers and fit less well into our electron density map. The overall architecture of the fungal DH is thus substantially more complex than in type-II FAS systems and resembles the hydratase fold known from fatty acid β oxidation.

The fungal NADPH-dependent ER is, in contrast to the other FAS systems, a FMN-containing oxidoreductase (*24*). ERs from different fungal and mycobacterial type-I FAS systems share high sequence conservation, but no homology to any structurally characterized protein has been observed, including to ER type-II enzymes. We therefore inspected currently unassigned electron density regions and discovered a TIM-barrel fold (Fig. 2D). Among 21 known TIM-barrel superfamilies, one is a class of FMN-dependent oxidoreductases (*25*). A good fit to the electron density map was obtained with the coordinates of spinach glycolate oxidase (*26*), which contains FMN as a cofactor in the catalytic center. Based on these observations, we propose that the FMN-binding part of fungal ER consists of a TIM-barrel fold. Adjacent to the FMN-binding pocket of the TIM barrel we observed an additional, predominantly α-helical domain, clearly different from a Rossmann fold, which may constitute the NADPH-binding part of the ER (fig. S2).

AT and MPT catalyze chemically similar acyl transfer reactions. Type-II acyl transferase sequences align to both the AT and MPT regions of the fungal FAS β chain, suggesting that the two enzymes share the same fold. Consequently, we could position the homologous malonyl transferase from *Streptomyces coelicolor* (*27*) (Fig. 3A) into two different regions of the FAS electron density map (Fig. 3B and fig. S3). The sequence alignment of bacterial type-II enzymes with the fungal FAS β chain positions the MPT immediately downstream of the DH domain, whereas the AT is separated from the DH by ~1150 residues (Fig. 1 and fig. S4). Fitting of the transacylase twice into the electron density map, without any prior assumptions, perfectly aligned the N terminus of one of the domains with the C terminus of the DH and identified it as MPT, and we assigned the other position to AT (Fig. 3C).

The terminal ACP and PT domains are connected to the body of the α chain via long, probably flexible, linkers. The ACP-flanking Pro/Ala/Gly-rich sequences are comparable to the linkers between moving domains of pyruvate dehydrogenase multienzyme complexes (*28*, *29*). As one might expect, at least for ACP, we could not identify electron density that would correspond to the ACP and PT structures in the 5 Å–resolution map. However, maps calculated at lower resolution reveal additional features in the interior and at the top of the particle (fig. S5A). The threefold related density at the apices might account for the PT, which forms homotrimers in the bacterial counterpart (fig. S5C) (*30*). The density observed in the interior may represent an attachment point to which ACP is flexibly tethered or a structural feature that facilitates substrate channeling (fig. S5B).

Overall architecture of the fungal FAS. The overall barrel shape of the FAS structure has a height of 260 Å and a width of 230 Å (Fig. 4A). Based on the placement of homologous structures, we present the three-dimensional domain organization within the multifunctional FAS assembly (Fig. 4C). The KS and KR dimers are located on the twofold axes and form the equatorial region of the barrel. In the connection emerging from the KR, we find the MPT. The second main portion of the density departing from the central region leads to the ER. Finally, AT and DH, together with parts of the ER, form the apical ring of the complex (Fig. 4, A and C). This domain distribution is consistent with studies of antibody cross-linking between FAS particles (*7*) and with chemical distance mapping of the subunits within one complex (*31*), and it corroborates the idea that the central part is built from α chains and that the β chains form the arches of the domes on either side of the particle (Fig. 4D).

The catalytic domains are embedded in a network of ''structural'' density. The central slice of the assembly forms a wheel-like structure (Fig. 4B). The hub of the wheel is built from six tightly packed α helices that emanate

toward the upper and lower chamber on each side of the wheel. Spokes radiate from the hub and join the domains arranged on the outer circular frame. The KR domains are connected to the center via single ~40 Å–long α helices, whereas the second type of spoke originating from the KS dimer is larger and of a more complex fold. We observed additional density at the periphery of the wheel, including two bundles of α helices: one bundle joining the KS with the KR and the other probably involved in KS dimerization. An even longer α helix of ~50 Å originates from the KR, runs in the longitudinal direction along the shell of the particle, and terminates in the middle of one of the two large openings of the barrel, where it contacts a currently unassigned domain (Fig. 4A).

The architecture of fungal FAS exemplifies how individual catalytic domains are assembled into a multifunctional enzyme. Gene fusion of individual proteins from a biosynthetic pathway into multidomain-coding sequences facilitates stoichiometric protein expression. The catalytic core domains of dissociative enzymes preserved their dimeric interactions in the multienzyme complex, as observed for KS and KR. We presume that the whole assembly gradually evolved by acquiring insertions and mutations in the linker regions at the periphery of the core domains. This created new protein interaction surfaces, resulting in higher order assemblies with improved catalytic properties.

The reaction chamber. Within fungal FAS, there are two identical large reaction chambers separated by the central wheel. Each of the two

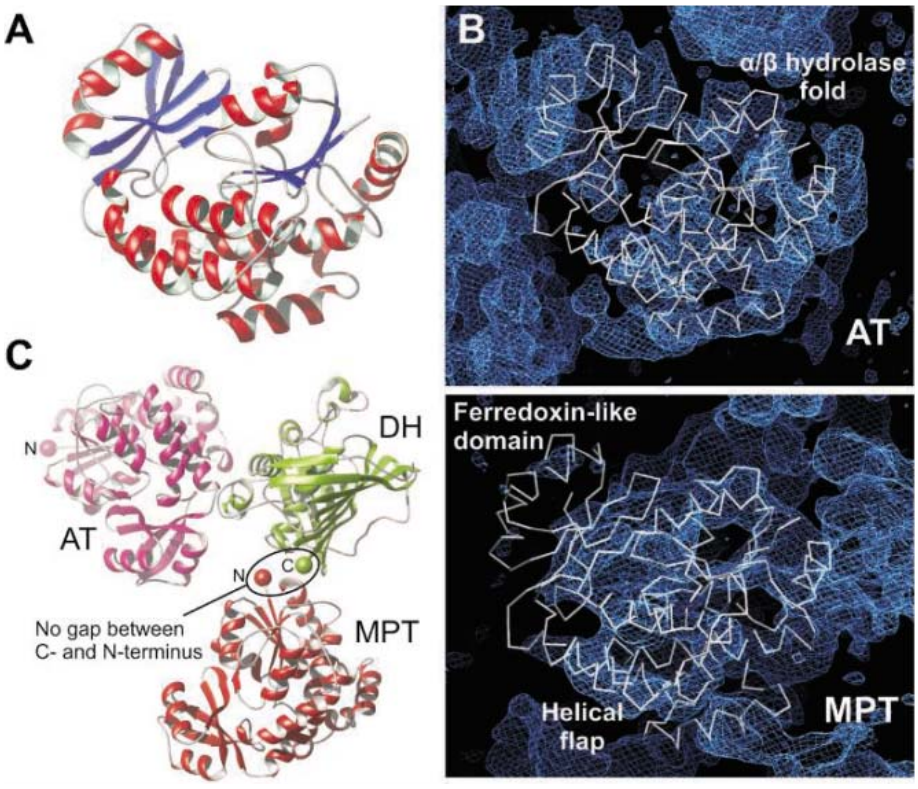

Fig. 3. Fitting of the homologous acyl transferase and functional assignment of electron densities to AT and MPT. (**A**) AT and MPT are homologous in sequence, catalyze related reactions, and adopt the same protein fold. (**B**) Fit of the domain into two different locations in the electron density map (PDB accession code 1NM2). (**C**) AT and MPT activities can be unambiguously assigned on the basis of the location of their N termini relative to the C terminus of the independently fitted DH and the corresponding order of the domains in the primary structure of the FAS β chain (Fig. 1 and fig. S4).

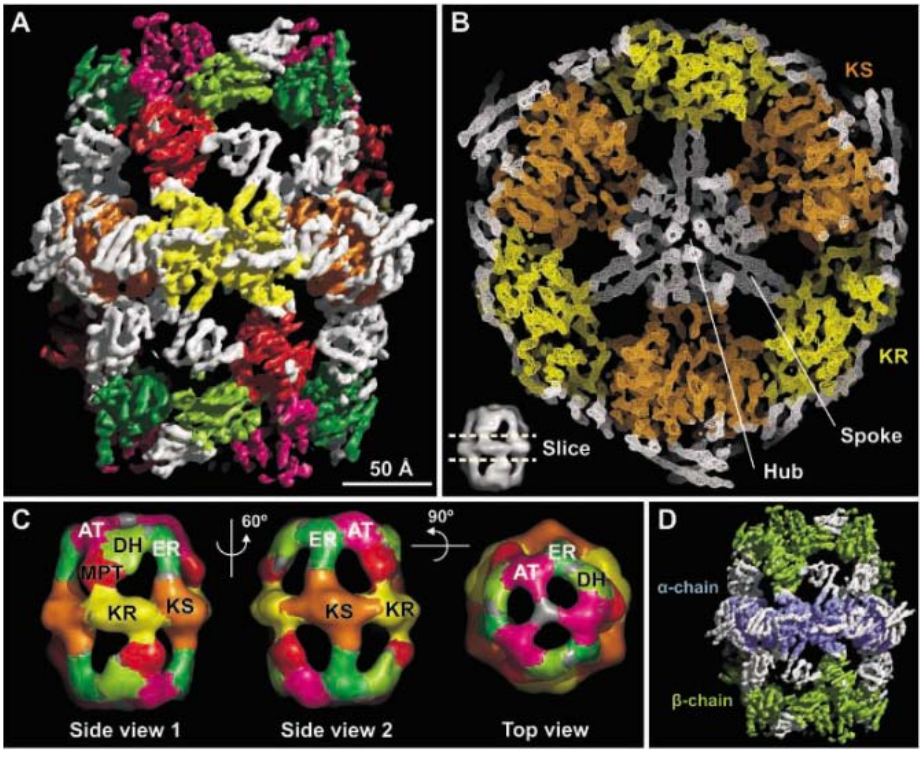

Fig. 4. The 5 Å electron density map of the fungal FAS. (**A**) Side view of the electron density along one of the twofold axes of FAS, contoured at 1.8σ. The density is colored according to the fitted domains, using the color scheme described in (C). Regions of electron density not corresponding to homologous domains are colored white, including the unassigned domain at the end of the 50 Å–long α helix that occludes one of the two large side openings. (**B**) Top view of the central wheel, which divides the interior of the FAS assembly into two reaction chambers. The KS and KR domains occupy only part of the electron density and are colored orange and yellow, respectively. Additional structural features involved in the formation of the FAS complex are shown in white. Spokes of electron density extend from the central hub of the wheel to the periphery. Bundles of α helices connect the KS and KR. (**C**) Arrangement of the different catalytic domains in the multienzyme complex. To illustrate the localization, the domains are mapped onto the cryoelectron microscopy reconstruction (*12*). KS is colored orange, KR yellow, MPT red, DH light green, ER dark green, and AT magenta. (**D**) Distribution of the α and β chains in the FAS complex. Electron density belonging to the α chain that forms the central wheel is shown in blue, the density of the β chain that folds into the arches on both sides of the FAS particle is in green, and the currently unassigned density is in white.

reaction chambers is equipped with three copies of a full set of catalytic domains required for fatty acid synthesis (Fig. 5A and fig. S6). The KS and KR dimers embedded in the base of the chambers expose one of their active sites to either the upper or the lower compartment. Similarly, the entrances to the active sites of the β-chain enzymes are accessible from the interior. Consequently, the reaction substrates and products enter and leave the reaction chambers by passive diffusion through the openings in the particle wall. With a diameter of up to ~25 Å, the holes are large enough to allow the passage of small molecules but provide a barrier to macromolecules.

Considering the internal arrangement of the catalytic sites and the cagelike structure of FAS, we propose that the ACPs are confined in the reaction chambers, because this would be the only mechanism by which ACP could accomplish substrate transfer. Large conformational changes within the FAS assembly that would alter the arrangement of catalytic sites during synthesis are not indicated by the structure; first, because of the fact that the particle maintains noncrystallographic symmetry to at least 5 Å resolution; and second, because most catalytic centers are embedded in a matrix of structural density. The observed distances between the active sites directly indicate that the 18 Å–long phosphopantetheine arm of ACP is much too short to reach all the catalytic centers by itself. Thus, ACP must move from one catalytic domain to the next during the reaction cycle, as postulated by Lynen (*1*) but not reflected in many textbooks. A mechanistically comparable process involving a swinging domain has also been proposed for pyruvate dehydrogenase complexes (*28*, *29*). Therefore, the long phosphopantetheine arm of ACP does not function as a swinging arm to pass substrates from domain to domain, but is rather required to deliver the acyl chain intermediates into the deep hydrophobic clefts of the active sites.

A comprehensive set of active sites is approximately equidistant from the internal structural feature (a possible attachment point for ACP) observed at lower resolution (Fig. 5B). The entrances to their catalytic centers are easily accessible from the interior of the reaction chamber (Fig. 5A). Without having the complete trace of the polypeptide chains, we cannot determine whether this combination of active sites originates from single or multiple α and β chains. Furthermore, the observed architecture suggests that ACP might also be able to reach alternative combinations of catalytic centers, which is in agreement with experiments showing in vitro complementation between mutant FAS complexes (*32*). The sequential transfer of ACP from one catalytic domain to the next is schematically illustrated in Fig. 5C. The acetyl primer substrate would be delivered to the KS from the AT located at the top. In this initial step, which is carried out only once during the reaction cycle, ACP must traverse

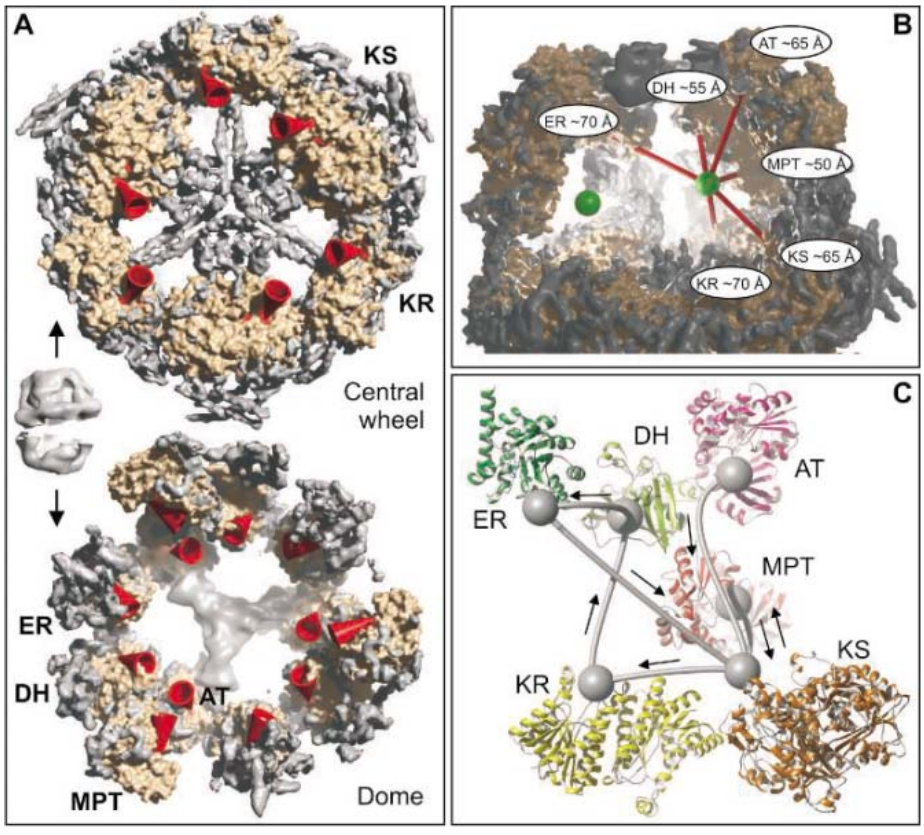

Fig. 5. (**A**) All active sites of the fungal FAS are oriented toward the interior of the reaction chamber. The dome (lower panel) is cut from the central wheel (upper panel) and flipped open. Fitted domains are colored in light brown and unassigned electron density is in gray. The trimeric connection at the apices of the particle observed in the 8 Å–resolution map is also shown in gray. Red cones indicate the entrances to the hydrophobic clefts that lead to the active sites. (**B**) Set of active sites in the reaction chamber with all enzymatic activities required for the fatty acid synthesis cycle. The view is into the reaction chamber, with one-third of the dome removed. Distances between the central structural feature (indicated by green spheres) and the active sites are indicated with red lines. (**C**) Schematic path of ACP, shown as a gray sphere, during substrate shuttling between the active sites.

the entire height of the reaction chamber. The MPT, on the other hand, is located directly above the KS, which may facilitate repetitive translocation of the elongation substrate. The condensed substrate from the KS would then be handed over to the KR, DH, and ER, following the figure-eight path depicted in Fig. 5C, until ACP brings the fully reduced intermediate back to the KS for the next elongation cycle.

Compartmentalization of fatty acid synthesis. Compartmentalization of fatty acid synthesis has the biological advantage of substrate channeling (*33*). In this process, the reaction intermediates covalently attached to ACP are prevented from diffusing into the bulk cytoplasm during transfer between the active sites. In addition, the concentration of ACP, and consequently also of all other active sites, within the chamber is approximately 1 mM, which ensures that none of the bimolecular reaction steps are rate limiting. This architectural solution has clear advantages over the FAS type-II system, with dissociated enzymes. *Escherichia coli*, for example, has to maintain very high concentrations of individual macromolecules that participate in these reactions, and ACP itself is the most abundant enzyme in the bacterial cytosol present at approximately 100 μM concentration (*34*, *35*). Therefore, compartmentalization in the fungal FAS increases the apparent concentration of ACP by an order of magnitude without the need to maintain high concentrations of FAS. In addition, the regulation of the reaction cycle can be more easily controlled: Only the FAS α and β chains, instead of eight individual proteins, have to be expressed in a concerted manner, and the basic capacity for the production of fatty acids can be controlled by the expression level of the FAS complex (*3*). However, FAS-dependent synthesis of full-length fatty acids depends on sufficient amounts of malonyl-CoA, the production of which is regulated by acetyl-CoA carboxylase (*36*, *37*).

The structure of the fungal FAS complex presented here provides first glimpses of the remarkable architectural principles that are used in megasynthases. Investigations of various aspects of FAS function through structure-based biochemical and genetic experiments can now

be performed. Starting from the results presented here on the fungal FAS and on the architecturally distinct mammalian FAS described in an accompanying paper (*38*), it may even be possible to ultimately obtain a detailed atomic model for both types of FAS assemblies.

References and Notes

1. F. Lynen, *Eur. J. Biochem.* **112**, 431 (1980).
2. S. J. Wakil, J. K. Stoops, V. C. Joshi, *Annu. Rev. Biochem.* **52**, 537 (1983).
3. E. Schweizer, J. Hofmann, *Microbiol. Mol. Biol. Rev.* **68**, 501 (2004).
4. S. Smith, A. Witkowski, A. K. Joshi, *Prog. Lipid Res.* **42**, 289 (2003).
5. S. W. White, J. Zheng, Y. M. Zhang, C. O. Rock, *Annu. Rev. Biochem.* **74**, 791 (2005).
6. F. Fichtlscherer, C. Wellein, M. Mittag, E. Schweizer, *Eur. J. Biochem.* **267**, 2666 (2000).
7. F. Wieland, E. A. Siess, L. Renner, C. Verfürth, F. Lynen, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5792 (1978).
8. J. K. Stoops *et al.*, *J. Biol. Chem.* **253**, 4464 (1978).
9. W. A. Hackenjos, H. J. Schramm, *Biol. Chem. Hoppe Seyler* **368**, 19 (1987).
10. J. K. Stoops, S. J. Kolodziej, J. P. Schroeter, J. P. Bretaudiere, S. J. Wakil, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6585 (1992).
11. S. J. Kolodziej, P. A. Penczek, J. P. Schroeter, J. K. Stoops, *J. Biol. Chem.* **271**, 28422 (1996).
12. S. J. Kolodziej, P. A. Penczek, J. K. Stoops, *J. Struct. Biol.* **120**, 158 (1997).
13. D. W. Brown, T. H. Adams, N. P. Keller, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 14873 (1996).
14. C. M. Watanabe, C. A. Townsend, *Chem. Biol.* **9**, 981 (2002).
15. C. P. Woloshuk, R. Prieto, *FEMS Microbiol. Lett.* **160**, 169 (1998).
16. K. Takayama, C. Wang, G. S. Besra, *Clin. Microbiol. Rev.* **18**, 81 (2005).
17. L. G. Dover, A. M. Cerdeño-Tarrága, M. J. Pallen, J. Parkhill, G. S. Besra, *FEMS Microbiol. Rev.* **28**, 225 (2004).
18. Materials and methods are described in the supporting material on *Science* Online.
19. J. G. Olsen *et al.*, *FEBS Lett.* **460**, 46 (1999).
20. M. Fisher *et al.*, *Struct. Fold. Design* **8**, 339 (2000).
21. K. M. Koski, A. M. Haapalainen, J. K. Hiltunen, T. Glumoff, *J. Mol. Biol.* **345**, 1157 (2005).
22. M. S. Kimber *et al.*, *J. Biol. Chem.* **279**, 52593 (2004).
23. D. Kostrewa, F. K. Winkler, G. Folkers, L. Scapozza, R. Perozzo, *Protein Sci.* **14**, 1570 (2005).
24. J. L. Fox, F. Lynen, *Eur. J. Biochem.* **109**, 417 (1980).
25. N. Nagano, C. A. Orengo, J. M. Thornton, *J. Mol. Biol.* **321**, 741 (2002).
26. Y. Lindqvist, C. I. Brändén, F. S. Mathews, F. Lederer, *J. Biol. Chem.* **266**, 3198 (1991).
27. A. T. Keatinge-Clay *et al.*, *Structure* **11**, 147 (2003).
28. R. N. Perham, *Biochemistry* **30**, 8501 (1991).
29. R. N. Perham, *Annu. Rev. Biochem.* **69**, 961 (2000).
30. K. D. Parris *et al.*, *Struct. Fold. Design* **8**, 883 (2000).
31. J. K. Stoops, S. J. Wakil, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4544 (1980).
32. K. Werkmeister, R. B. Johnston, E. Schweizer, *Eur. J. Biochem.* **116**, 303 (1981).
33. X. Huang, H. M. Holden, F. M. Raushel, *Annu. Rev. Biochem.* **70**, 149 (2001).
34. M. S. Davis, J. E. Cronan Jr., *J. Bacteriol.* **183**, 1499 (2001).
35. L. Tang, A. C. Weissborn, E. P. Kennedy, *J. Bacteriol.* **179**, 3697 (1997).
36. T. Hori, N. Nakamura, H. Okuyama, *J. Biochem.* (*Tokyo*) **101**, 949 (1987).
37. M. Sumper, D. Oesterhelt, C. Riepertinger, F. Lynen, *Eur. J. Biochem.* **10**, 377 (1969).
38. T. Maier, S. Jenni, N. Ban, *Science* **311**, 1258 (2006).

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Supporting Online Material
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Materials and Methods
Figs. S1 to S6
Tables S1 and S2
References

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Grain Size–Sensitive Creep in Ice II

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Rheological experiments on fine-grained water ice II at low strain rates reveal a creep mechanism that dominates at conditions of low stress. Using cryogenic scanning electron microscopy, we observed that a change in stress exponent from 5 to 2.5 correlates strongly with a decrease in grain size from about 40 to 6 micrometers. The grain size–sensitive creep of ice II demonstrated here plausibly dominates plastic strain at the low-stress conditions in the interior of medium- to large-sized icy moons of the outer solar system.

High-pressure phases of water ice are major constituents of the interiors of low-density icy moons with radii of >700 km, namely Ganymede and Callisto (Jupiter); Titan, Rhea, and Iapetus (Saturn); Titania and Oberon (Uranus); and Triton (Neptune) (*1*). These moons were warmed by accretional heating and are often internally heated, either by tidal stresses of a nearby giant planet or by radioactive decay of a rocky component. The rheology of ice, as described by the relationship $\dot{\varepsilon} \propto f(\sigma)$ between a strain rate $\dot{\varepsilon}$ and differential stress σ, can control the thermal evolution and internal dynamics of icy moons (*2*–*6*). Typically, in crystalline solids the stress sensitivity is described by a power law, $\dot{\varepsilon} \propto \sigma^n$, where the stress exponent n is a constant. Creep experiments have been carried out at pressure-temperature (*P*-*T*) conditions relevant to the interiors of icy moons to determine the flow law of several high-pressure phases (*7*–*12*). In those studies, differential stresses were relatively high and the grain size of samples was not controlled or examined. In all measurements to date on the high-pressure phases of ice, the stress exponent is relatively large ($n > 4$), which implies dominance of a grain size–insensitive (GSI) deformation mechanism such as dislocation creep (*13*).

Dislocation creep may not be the dominant mechanism of deformation at the low levels of stress ($\sigma < 0.1$ MPa) expected in the convecting interiors of icy moons (*14*, *15*). According to the flow law, as stress decreases, mechanisms of lower n [particularly the contribution of grain size–sensitive (GSS) processes, such as diffusion creep and superplasticity (*13*, *16*)] contribute proportionally more to the total strain rate than do mechanisms of higher n. For planetary applications, it is essential to identify and characterize creep mechanisms that may dominate at low stresses. We report results of creep experiments with the use of fine-grained ice II at low–strain rate conditions to 10^{-8} s^{-1}. We synthesized fine-grained ice II by rapid cycling of the transformation from ice II to ice I (II-I transformation), followed by repressurization, and observed and measured ice II grain size with the use of a cryogenic scanning electron microscope (SEM). Using these techniques, we found a low-n creep mechanism that is weaker than GSI creep in ice II and dominant at lower stresses and finer grain sizes.

Synthesis of the ice II samples and subsequent creep experiments were carried out in a cryogenic gas-medium deformation apparatus (*10*, *17*). The starting ice I (*18*) samples with a grain size of about 250 μm were transformed to ice II by pressurizing to ~300 MPa at a constant pumping rate,

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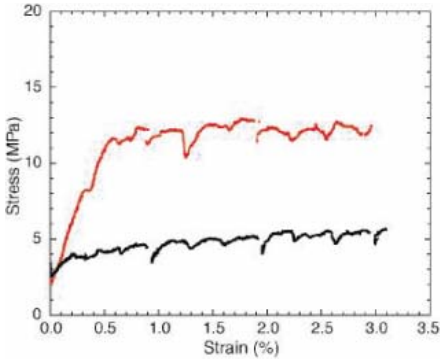

Fig. 1. Stress-strain curve of ice II made by the single (red, run 516) and triple (black, run 511) I-II transformation obtained at a pressure of 200 MPa, temperature of 200 K, and constant strain rate of around 3.9×10^{-8} s^{-1} (tables S1 and S2). Breaks in the curves represent periodic unloading of the sample in order to rezero the internal force gauge. Fluctuations in the stress-strain curve are mainly due to temperature fluctuations when exchanging liquid nitrogen tanks.

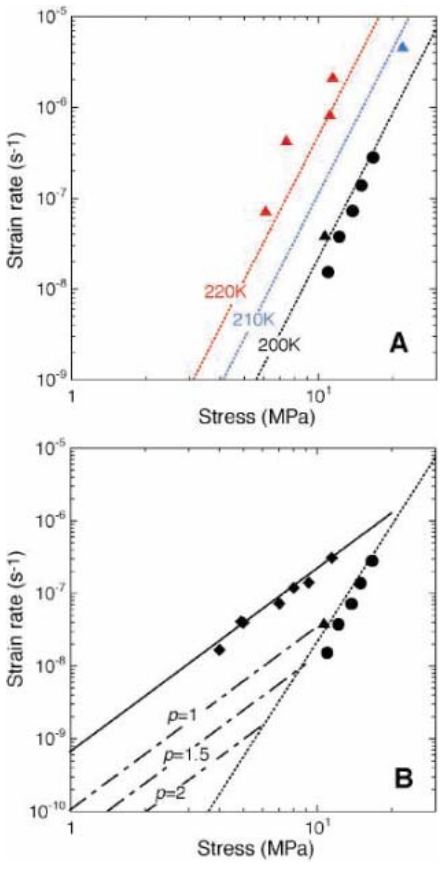

Fig. 2. Creep results for ice II with single (circle), double (triangle), and triple (diamond) I-II transformations at temperatures of 200 K (black), 210 K (blue), and 220 K (red). The grain sizes of ice II with the single and triple I-II transformations are estimated to be 38 ± 14 and 6 ± 2 μm, respectively (tables S1 and S2). Data at various pressures are adjusted to P = 200 MPa using an activation volume V^* = 7 cm^3/mol (*10*). Dotted lines are the flow law with the stress exponent n = 5.3 previously obtained (*10*). (**A**) Creep data for ice II with single and double I-II transformations are consistent with the n = 5.3 rheology at 200 to 220 K. (**B**) Creep data for ice II with triple I-II transformations indicate lower flow strength and a smaller stress exponent of n = 2.5 (solid line) at 200 K. The dashed lines show the n = 2.5 rheology at a grain size of 38 μm (i.e., that of ice II after a single I-II transformation) assuming grain-size exponents of p = 1, 1.5, and 2. Plotted data are listed in table S2.

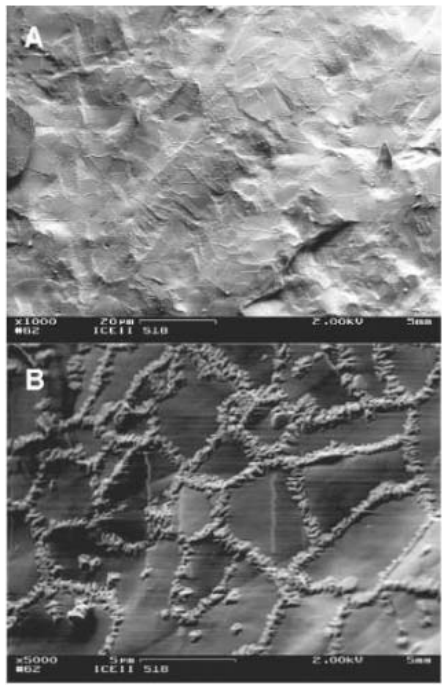

Fig. 3. SEM images of fractured fresh surfaces of polycrystalline ice II (*23*) showing ice II grain boundaries decorated by ice I grains. (**A**) Wide view of the sample. (**B**) Enlarged image of (A). The ice I stands in raised relief relative to the ice II due to the volumetric expansion to the lower-density phase. This ice II sample (run 518) was made by the triple I-II transformations and plastically deformed to 6% strain. After the creep experiment, the sample was partially back transformed to ice I and then quenched (tables S1 and S2) (*22*).

ordinarily 20 to 30 MPa/min in the absence of transformation, at constant T in the range of 174 to 219 K. Large overpressures are needed at lower temperatures to initiate the I-II transformation (fig. S1). After complete I-II transformation, the pressure was vented from 300 to 30 MPa in about 5 s, which is expected on the basis of earlier experiments that produced ice I with a grain size of about 10 μm (*19*, *20*). The transformation to ice II was then repeated, and in some samples a second rapid venting to ice I was performed, followed by a third transformation to ice II (table S1). We carried out deformation experiments on the samples after the first, second, or third I-II transformation (table S2). In one case (run 511), we deformed the sample after both the second and third transformations.

Six ice I samples were converted to ice II (table S1) and plastically deformed in compression at constant strain rates of 1.4×10^{-8} to 4.3×10^{-6} s^{-1} at P = 200 to 250 MPa and T = 200 to 220 K. Each run consisted of several fixed-condition steps of 1 to 3% strain, giving a total of 18 measurements of steady-state flow strength. The measured flow strength ranged from 4.0 to 22 MPa and total strains ranged from 3 to 17% (table S2). Figure 1 shows clear differences in creep behavior between ice II samples made by single and triple I-II transformations. At a constant strain rate of 3.9×10^{-8} s^{-1} (~1% strain per 3 days) and T = 200 K, the flow strength of the triple-transformation sample is less than half that of the single-transformation sample.

Creep results are summarized in Fig. 2. We expand the rheological relationship given above as $\dot{\varepsilon} = A\sigma^n d^{-p}\exp[-(E^* + PV^*)/RT]$, where d is grain size (diameter), and A, p, E^*, and V^* are the flow constants: preexponential factor, grain-size exponent, activation energy, and activation volume, respectively. Adjusting the data at various pressures to 200 MPa using V^* = 7 cm^3/mol (*10*), the creep data for ice II samples with single and double I-II transformations are consistent with the flow law previously obtained at σ > 20 MPa, for which n = 5.3 (*10*), to a lowest stress of 6.1 MPa (Fig. 2A). Ice II made by triple I-II transformation, however, shows a different rheology with a stress exponent n = 2.5 at σ = 4.0 to 11 MPa (Fig. 2B).

Ice II can be metastably present at 0.1 MPa and T < 120 K (*21*). After our creep experiments, samples were cooled to <100 K at $P \approx$ 200 MPa and then depressurized. The right-cylindrical shape of the samples (e.g., fig. S2) suggests spatial uniformity of all the processes to which the samples were subjected, including multiple transformations and several deformation steps.

The indium jackets encapsulating the samples during testing provide replicas of the outer surface of the ice samples (*11*). From SEM observations of these replicas, we estimate that the grain size of ice II samples after a single I-II transformation is 38 ± 14 μm (table S1). For the multiply transformed ice II samples, grain diameters were apparently too small for fair replication. For these samples, we decorated ice II grain boundaries by partial back transformation to ice I (*22*) and then observed the samples directly by cryogenic SEM (*23*). The networks formed by back transformation (Fig. 3) are so strongly reminiscent of grain boundaries that, in the absence of direct phase identification, we take them to be ice I grains heterogeneously nucleated on ice II grain boundaries. Furthermore, the raised relief of the ice I grains relative to ice II is in accordance with its lower density; to verify this, one sample of ice II was warmed

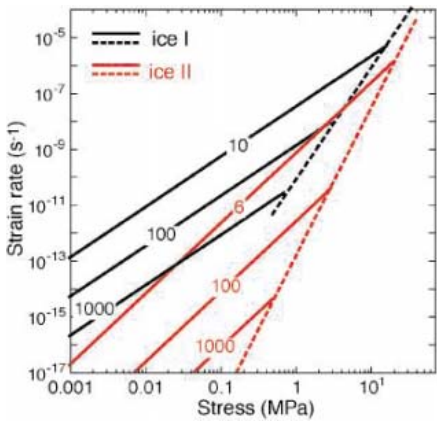

Fig. 4. Comparison of ice I (black) and ice II (red) rheology in both the GSI (dotted lines) and GSS (solid lines) creep regimes at 200 MPa and 200 K. Numbers are grain size (μm). Creep data for ice I were taken from previous studies (*26*, *27*). The activation volume for GSS creep was taken as that for GSI creep in both ice I and ice II (*10*). GSS creep of ice II for grain sizes 100 and 1000 μm are plotted on the basis of the creep data obtained for 6 μm and assuming a grain-size exponent $p = 2$.

to above 120 K under the SEM beam. We observed the expected volumetric expansion accompanying full reversion to ice I. The size of ice II grains thus identified is 6 ± 2 μm in the sample with the triple I-II transformations and measurably smaller than the approximate 38-μm grain size achieved by single I-II transformation (table S1). The correlation between number of transformation cycles and rheology thus suggests that grain size and rheology are strongly correlated.

Another effect of repeated pressure cycling is a change in the character of the I-II transformation. Given the ~21% volume reduction of the sample (*24*), P can sometimes be higher before the transformation than afterwards. We detected the I-II transformation during pressurization by deviations from the normal P-versus-time trend at a steady rate of pumping. These deviations become increasingly sharp with repeated phase transformations (fig. S3 and table S1). If the sharpness is related inversely to grain size as the SEM observations suggest, then the grain size after the second I-II transformation, which was not observed by SEM, might be expected to be between 38 and 6 μm.

We demonstrate a change from a GSI to a weaker GSS rheology at low stresses and finer grain sizes in ice II. As Fig. 2B shows, at 200 MPa and 200 K, the fine-grained ice II of $d \approx 6$ μm is much weaker than ice II of $d \approx 40$ μm. Furthermore, the finer-grained material has a distinctly lower stress exponent than does the coarse-grained material: $n = 2.5$ versus $n = 5.3$. We estimate roughly a grain-size exponent $p > 1.5$ on the basis of the dashed lines in Fig. 2B; if p were <1.5, the lowest circle in Fig. 2B (ice II of $d = 38$ μm, the single I-II transition, at the lowest strain rate of 1.6×10^{-8} s^{-1}) would reflect the transition to GSS creep and would be shifted to lower stresses.

Ice II with grain size of about 15 μm shows that grain size induced weakening (*19*), which is consistent with our results. GSS creep has been reported in ice I at ambient conditions (*25*, *26*). The flow law of GSS creep found in various materials including ice I is generally characterized by a smaller stress exponent $n \approx 2$ instead of $n \approx 5$ for dislocation creep, and a grain-size exponent of $p \approx 2$ (*13*, *16*). GSS creep in ice II with stress exponent $n = 2.5$ is consistent with these previous studies.

Figure 4 is a comparison of GSI and GSS rheologies for both ice I and ice II at 200 MPa and 200 K. For a given grain size, ice II is stronger than ice I for both creep mechanisms. For $d \approx 10$ μm, a transition from GSI creep to GSS creep occurs at $\sigma \approx 10$ MPa in both ice I and ice II. Assuming a typical grain-size exponent of $p = 2$ for GSS creep in ice II, extrapolation of the flow law to more planetary-relevant grain sizes of $d = 1$ and 10 mm suggests that GSS creep becomes dominant at $\sigma < 0.5$ and 0.1 MPa, respectively; for $p = 1.5$, the transition to GSS creep in ice II occurs at $\sigma = 1$ and 0.5 MPa, respectively.

The stress levels in the density-driven, convecting interiors of medium- and large-size icy moons have been estimated to be on the order of 0.01 MPa (*14*) and 0.1 MPa (*15*), respectively. Therefore, it is likely that both ice I and ice II plastically deform by the GSS creep mechanisms in the interior of icy moons when the grain size is less than 10 mm. In Fig. 4, for example, at a stress of 0.1 MPa and grain size of 1 mm, the viscosities $(\sigma/3\dot{\varepsilon})$ for GSI creep in ice II, GSS creep in ice II, and GSS creep in ice I are 5.2×10^{22}, 4.7×10^{20}, and 4.4×10^{16} Pa·s, respectively, at a pressure of 200 MPa and temperature of 200 K. The viscosity contrast between ices I and II at the I-II transition depth will therefore be about four orders of magnitude (ice II is the stronger of the two) if the ice II is deforming in GSS creep and six orders of magnitude if GSS creep is suppressed. The difference in flow patterns and heat transfer in the interiors will be substantial. Thus, although further quantitative investigations of the grain-size and temperature dependence are needed, the GSS creep of ice II demonstrated in this study is a possible candidate for a flow mechanism that controls the thermal evolution and internal dynamics of medium- and large-size satellites of the outer planets.

References and Notes

1. D. A. Rothery, *Satellites of the Outer Planets* (Oxford Univ. Press, New York, 1999).
2. G. L. Consolmagno, J. S. Lewis, *Icarus* **34**, 280 (1978).
3. R. T. Reynolds, P. M. Cassen, *Geophys. Res. Lett.* **6**, 121 (1979).
4. J. P. Poirier, *Nature* **299**, 683 (1982).
5. G. Schubert, T. Spohn, R. T. Reynolds, in *Satellites* (Univ. Arizona Press, Tucson, AZ, 1986), pp. 242–292.
6. W. B. McKinnon, in *Solar System Ices* (Kluwer, Dordrecht, Netherlands, 1998), pp. 525–550.
7. J. P. Poirier, C. Sotin, J. Peyronneau, *Nature* **292**, 225 (1981).
8. C. Sotin, P. Gillet, J. P. Poirier, in *Ices in the Solar System*, J. Klinger *et al.*, Eds. (D. Reidel, Hingham, MA, 1985), pp. 109–118.
9. C. Sotin, J. P. Poirier, *J. Phys.* **48**, 233 (1987).
10. W. B. Durham, S. H. Kirby, H. C. Heard, L. A. Stern, C. O. Boro, *J. Geophys. Res.* **93**, 10191 (1988).
11. W. B. Durham, L. A. Stern, S. H. Kirby, *J. Geophys. Res.* **101**, 2989 (1996).
12. K. Echelmeyer, B. Kamb, *Geophys. Res. Lett.* **13**, 693 (1986).
13. J. P. Poirier, *Creep of Crystals* (Cambridge Univ. Press, Cambridge, 1985).
14. K. Ellsworth, G. Schubert, *Icarus* **54**, 490 (1983).
15. G. Schubert, D. J. Stevenson, K. Ellsworth, *Icarus* **47**, 46 (1981).
16. J. W. Edington, K. N. Melton, C. P. Cutler, *Prog. Mater. Sci.* **21**, 61 (1976).
17. H. C. Heard *et al.*, in *Geophys. Monogr. Am. Geophys. Union 56*, A. G. Duba *et al.*, Eds. (American Geophysical Union, Washington, DC, 1990), pp. 225–228.
18. Starting materials are molded synthetic ice I aggregates, 63 mm long and 25 mm in diameter with a grain size of about 250 μm (*19*). The starting sample has a uniform texture with virtually no porosity and a random grain orientation.
19. L. A. Stern, W. B. Durham, S. H. Kirby, *J. Geophys. Res.* **102**, 5313 (1997).
20. W. B. Durham, L. A. Stern, S. H. Kirby, *J. Geophys. Res.* **106**, 11031 (2001).
21. J. E. Bertie, L. D. Calvert, E. Whalley, *J. Chem. Phys.* **38**, 840 (1963).
22. After the creep experiments in the stability field of ice II, the pressure was slowly decreased to the ice I stability field at the rate of about 7 MPa/min at constant temperature of 200 K. When the back II-I transition started at about 140 MPa, which was identified from the slight slowing of the rate of pressure decrease, the pressure was increased back to that at the I-II phase boundary. The sample length, which can be measured by the piston contact to the sample (*11*), showed slight lengthening due to the partial back transformation. The sample was then cooled along the I-II phase boundary to <100 K, depressurized, removed from the vessel, and stored at 77 K.
23. SEM observations were conducted on the uncoated sample with a cryogenic stage at temperatures of less than 100 K and low accelerating voltage of 2 kV with the use of a LEO 982 field emission SEM. Before imaging, the sample was fractured with a cold blade at 100 K under vacuum to produce fresh surfaces that were not contaminated by surface condensation.
24. N. H. Fletcher, in *The Chemical Physics of Ice* (Cambridge Univ. Press, Cambridge, 1970), p. 57.
25. D. L. Goldsby, D. L. Kohlstedt, *Scr. Mater.* **37**, 1399 (1997).
26. D. L. Goldsby, D. L. Kohlstedt, *J. Geophys. Res.* **106**, 11017 (2001).
27. W. B. Durham, S. H. Kirby, L. A. Stern, *J. Geophys. Res.* **102**, 16293 (1997).

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Supporting Online Material

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Figs. S1 to S3
Tables S1 and S2

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Giant Electrocaloric Effect in Thin-Film $PbZr_{0.95}Ti_{0.05}O_3$

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An applied electric field can reversibly change the temperature of an electrocaloric material under adiabatic conditions, and the effect is strongest near phase transitions. We demonstrate a giant electrocaloric effect (0.48 kelvin per volt) in 350-nanometer $PbZr_{0.95}Ti_{0.05}O_3$ films near the ferroelectric Curie temperature of 222°C. A large electrocaloric effect may find application in electrical refrigeration.

There has been increasing interest in alternative cooling technologies over the past decades. First, it is important to reduce greenhouse gases that are used heavily in domestic and industrial refrigeration. Second, higher current densities in integrated circuits will impose higher demands on cooling systems that cannot be exclusively met by the current fan-based solutions. The electrocaloric (EC) effect is a change in the temperature of a material upon the application or withdrawal of an electric field under adiabatic conditions. It generated great interest (*1*–*4*) in the 1960s to 1970s but has not been exploited commercially because the reported EC effects were small. For example, bulk $Pb_{0.99}Nb_{0.02}(Zr_{0.75}Sn_{0.20}Ti_{0.05})_{0.98}O_3$ shows the highest EC effect measured so far, with direct measurements giving a peak value of 2.5 K in 750 V (*1*). Here, we report a giant EC effect of 12 K in 25 V in thin films of Zr-rich $Pb(Zr,Ti)O_3$ (PZT).

Understanding of the mechanisms underlying the EC effect is not yet established. Three textbooks on ferroelectricity differ on the macroscopic physics of the EC effect (*5*–*7*). Fatuzzo and Merz (*5*) argue that the EC effect only occurs above the phase transition (Curie) temperature T_C, where the polarization P is finite in the presence of the applied electric field E. Mitsui, Tatsuzaki, and Nakamura (*6*) argue that the EC effect can only occur below T_C, where the spontaneous value of P changes with temperature. Jona and Shirane (*7*) disagree with both (*5*) and (*6*) and assert that the effect occurs both above and below T_C but is larger above. More generally, microscopic models of ferroelectrics are not well established. For example, it is only in the last decade that a fully quantum-mechanical treatment has been available (*8*, *9*).

It is plausible that thin films of Zr-rich PZT show promising EC effects, because the converse effect of pyroelectricity is pronounced and forms the basis of infrared detectors (*10*). Both Zr-rich PZT and the more common compositions such as $PbZr_{0.52}Ti_{0.48}O_3$ are used as capacitors because of their high dielectric constants (*11*) and also as high-strain actuators/transducers and prototype microelectromechanical systems because of their piezoelectric properties (*11*). However, the potential for PZT thin films in cooling applications has not been considered.

Bulk $Pb(Zr_{0.95}Ti_{0.05})O_3$ is an orthorhombic antiferroelectric at room temperature. On heating to ~120°C, this structure transforms to a rhombohedral ferroelectric phase. There is substantial thermal hysteresis in this antiferroelectric to ferroelectric transition, which on cooling occurs at ~80°C. The structure transforms to cubic paraelectric above 242°C. This is a first-order phase transition with a Curie temperature of T_o = 225°C, extrapolated linearly from the inverse dielectric susceptibility (*12*). The rhombohedral to paraelectric transition at this composition is close to a tricritical point at $PbZr_{0.94}Ti_{0.06}O_3$, where its character changes from first to second order (*13*). These transition temperatures, which were taken from single crystals and high-purity ceramics, differ somewhat from much earlier data (*14*) in which the sample purity was not as good. The EC effect could not be predicted from the literature, as there is no data for Zr-rich PZT thin films at the high temperatures and high electric fields of interest.

Sol-gel ~350-nm PZT films were prepared, characterized, and measured as described in (*15*). Electrical hysteresis measurements were made roughly every 15°C in the temperature range 35° to 280°C, on cooling to minimize reductions in P due to fatigue. Representative plots of $P(E)$ are shown in Fig. 1, A to D. The real part of the effective dielectric constant ε and loss tangent, measured every 1°C on cooling, each show a broad peak associated with the ferroelectric-paraelectric transition at T_C = 222°C (Fig. 1E), but no peaks corresponding to the antiferroelectric-ferroelectric

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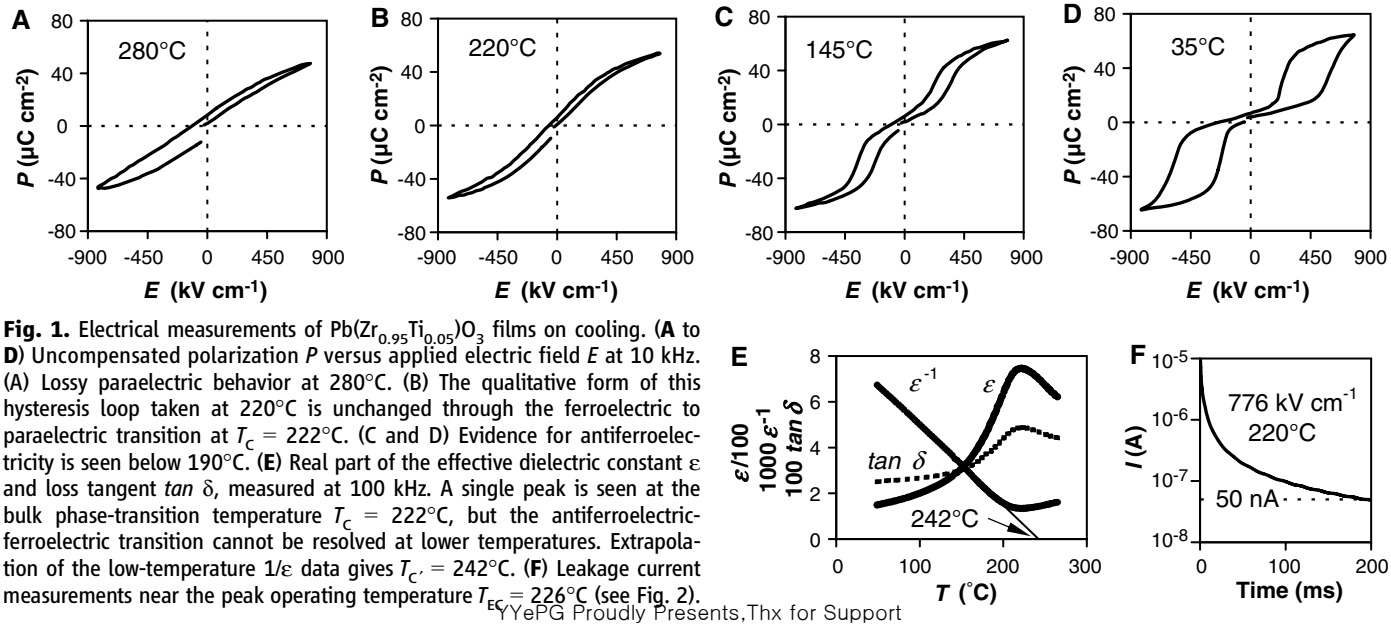

Fig. 1. Electrical measurements of $Pb(Zr_{0.95}Ti_{0.05})O_3$ films on cooling. (**A** to **D**) Uncompensated polarization P versus applied electric field E at 10 kHz. (A) Lossy paraelectric behavior at 280°C. (B) The qualitative form of this hysteresis loop taken at 220°C is unchanged through the ferroelectric to paraelectric transition at T_C = 222°C. (C and D) Evidence for antiferroelectricity is seen below 190°C. (**E**) Real part of the effective dielectric constant ε and loss tangent *tan* δ, measured at 100 kHz. A single peak is seen at the bulk phase-transition temperature T_C = 222°C, but the antiferroelectric-ferroelectric transition cannot be resolved at lower temperatures. Extrapolation of the low-temperature 1/ε data gives $T_{C'}$ = 242°C. (**F**) Leakage current measurements near the peak operating temperature T_{EC} = 226°C (see Fig. 2).

transition can be resolved. This broadness is typical of thin films and is likely due to interfacial strain, scalar concentration gradients, or other forms of microscopic variability (*16*). Extrapolation of the low-temperature $1/\varepsilon$ data to zero gives $T_{C'}$ = 242°C, corresponding to the bulk value (*12*).

Reversible adiabatic changes in temperature ΔT for a material of density ρ with heat capacity C are given (*17*) by

$$\Delta T = -\frac{1}{C\rho}\int_{E_1}^{E_2} T\left(\frac{\partial P}{\partial T}\right)_E dE, \qquad (1)$$

assuming the Maxwell relation $(\partial P/\partial T)_E = (\partial S/\partial E)_T$. Values of $\partial P/\partial T$ were obtained from fourth-order polynomial fits to $P(T)$ data (Fig. 2, inset) extracted from $P(E)$. Fatigue may only reduce our values of $|\partial P/\partial T|$ because the data were taken on cooling such that P increased in successive hysteresis measurements. In the temperature range of interest, the heat capacity C = 330 J K^{-1} kg^{-1} remains constant for Zr-rich PZT films, and the peak associated with the transition is <10% of the background (*18*, *19*). Assuming a constant value of C despite a ~50% peak (*17*) resulted in excellent agreement with direct EC measurements of ΔT in bulk $Pb_{0.99}Nb_{0.02}(Zr_{0.75}Sn_{0.20}Ti_{0.05})_{0.98}O_3$ (*1*). A value of ρ = 8.3 g cm^{-3} reported for the similar compound $(Pb,Zr,Sn)TiO_3$ was used here (*17*). The lower integration limit E_1 = 295 kV cm^{-1} was set deliberately high to avoid the antiferroelectric regime (at low fields) (Fig. 1, C and D), which ensures that $\partial P/\partial T < 0$. The upper integration limit E_2 = 776 kV cm^{-1} represents the maximum field at which a consistent data set could be obtained.

EC temperature changes obtained with Eq. 1 are presented in Fig. 2, where the peak change (12 K in a maximum applied voltage of 25 V, i.e., 0.48 K V^{-1}) at T_{EC} = 226°C exceeds the previous best results obtained (*1*) in bulk $Pb_{0.99}Nb_{0.02}(Zr_{0.75}Sn_{0.20}Ti_{0.05})_{0.98}O_3$ (2.5 K in 750 V, i.e., 0.003 K V^{-1}) at T_{EC} = 162°C. By resorting to a thin-film geometry, we were able to apply electric fields that exceed bulk breakdown fields (~50 kV cm^{-1}) by an order of magnitude (*20*). Indeed, our maximum field change ($E_2 - E_1$ = 480 kV cm^{-1}) is 16 times as large as the 30 kV cm^{-1} applied in (*1*). However, the relevant figure of merit for applications depends on the external voltage applied rather than the internal field generated. Films also offer a wide range of possible working temperatures (Fig. 2) associated with the broad phase transitions that they display (Fig. 1E).

The use of reversible thermodynamics (Eq. 1) to determine the above result is justified in view of the relatively small hysteresis losses. Our peak EC temperature change of ΔT = 12 K, determined with E_1 = 295 kV cm^{-1} and E_2 = 776 kV cm^{-1}, represents a peak energy change $C\Delta T$ = 4.02 kJ kg^{-1}. The corresponding hysteresis loss was 4% of this figure, as determined from the area of the 220°C hysteresis loop taken near the peak T_{EC} = 226°C (Fig. 1B) between the same values of E_1 and E_2. Therefore, hysteresis losses have the potential to reduce our peak EC temperature change by only ~0.5 K.

Leakage currents were investigated near the peak EC temperature in the maximum field employed (Fig. 1F). The observed transients persist up to 200 ms, beyond which breakdown occurs. Therefore, our figure of 50 nA is an upper bound for the steady-state leakage current. This value yields negligible Joule heating (~10^{-3} K) and does not affect $P(E)$ because currents of hundreds of μA are required to switch the measured polarizations at 10 kHz.

There is plenty of scope for optimizing EC effects. For theoretical insights about the models (*5*–*7*) discussed earlier, it will be necessary to avoid the broad transitions seen in films, for example, by nanopatterning single crystals (*16*). We now discuss various materials improvements: (i) The use of oxide electrode such as $SrRuO_3$ should increase breakdown fields and reduce fatigue (*21*, *22*). (ii) Aliovalent doping with, for example, A-site La^{3+} or B-site Mn^{3+} will improve fatigue properties (*10*, *23*). (iii) The partial substitution of Sn for Zr (*1*) or Sr for Pb (*24*) will lower T_{EC} toward room temperature. (iv) The development of ferroelectrics that contain Bi rather than toxic Pb is an active area of research that could be relevant (*25*). (v) The introduction of crystallographic texture is desirable because materials of interest are anisotropic and low-angle grain boundaries enhance thermal conductivity.

We hope that the EC effect demonstrated here will inspire commercial applications. For example, the EC effect could provide cooling solutions for electronic components such as computer chips. The converse pyroelectric effect could be used, for example, to recover useful electrical power from waste heat. Given that primary pyroelectricity is larger than its secondary counterpart, the effects reported here in clamped thin films would be enhanced in stress-free environments (*26*, *27*).

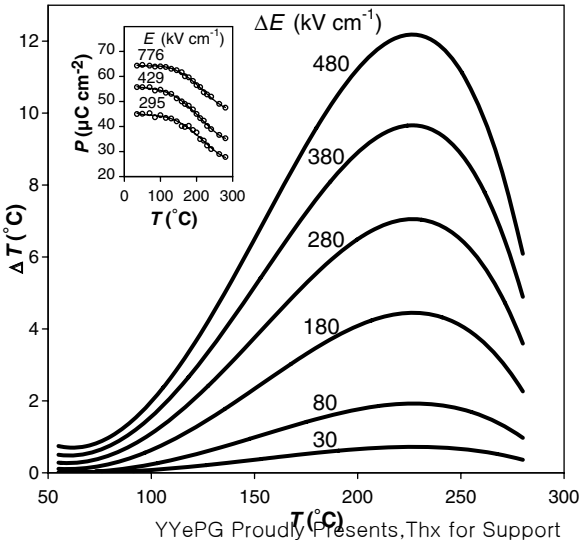

Fig. 2. Electrocaloric temperature changes ΔT due to applied ΔE. Calculations were performed using Eq. 1 with selected values of $\Delta E = E_2 - E_1$, where E_2 = 776 kV cm^{-1}. The peak value of ΔT = 12 K occurs in ΔE = 480 kV cm^{-1} at T_{EC} = 226°C, where $|\partial P/\partial T|$ is maximized. (**Inset**) $P(T)$ at selected applied fields E. The lines represent fourth-order polynomial fits to data extracted from the upper branches of 19 hysteresis loops in $E > 0$. Four of the 19 loops are shown in Fig. 1, A to D.

References and Notes

1. B. A. Tuttle, D. A. Payne, *Ferroelectrics* **37**, 603 (1981).
2. P. D. Thacher, *J. Appl. Phys.* **39**, 1996 (1968).
3. W. N. Lawless, *Phys. Rev. B* **16**, 433 (1977).
4. B. A. Strukov, *Sov. Phys. Crystallogr.* **11**, 757 (1967).
5. E. Fatuzzo, W. J. Merz, *Ferroelectricity* (North-Holland, Amsterdam, 1967).
6. T. Mitsui, I. Tatsuzaki, E. Nakamura, *Introduction to the Physics of Ferroelectricity* (Gordon and Breach, London, 1976).
7. F. Jona, G. Shirane, *Ferroelectric Crystals* (McMillan, NY, 1962).
8. R. Resta, *Rev. Mod. Phys.* **66**, 899 (1994).
9. R. D. Kingsmith, D. Vanderbilt, *Phys. Rev. B* **47**, 1651 (1993).
10. Q. Zhang, R. W. Whatmore, *J. Appl. Phys.* **94**, 5228 (2003).
11. W. Y. Pan, C. Q. Dam, Q. M. Zhang, L. E. Cross, *J. Appl. Phys.* **66**, 6014 (1989).
12. R. W. Whatmore, thesis, Cambridge University (1977).
13. R. W. Whatmore, R. Clarke, A. M. Glazer, *J. Phys. C: Solid State Phys.* **11**, 3089 (1978).
14. E. Sawaguchi, *J. Phys. Soc. Jpn.* **8**, 615 (1953).
15. Materials and methods are available as supporting material on *Science* Online.
16. M. M. Saad *et al.*, *J. Phys. Cond. Matter* **16**, L451 (2004).
17. B. A. Tuttle, thesis, University of Illinois at Urbana–Champaign (1981).
18. S. T. Davitadze, thesis, Lomonosov Moscow State University (2003).
19. G. Suchanek *et al.*, *Mat. Res. Soc. Proc.* **718**, D8.4.1 (2002).
20. J. F. Scott, *Ferroelectric Memories* (Springer, Berlin, 2000).
21. U. Ellerkmann *et al.*, *Integrated Ferroelectrics* **52**, 63 (2003).
22. K. Maki *et al.*, *Integrated Ferroelectrics* **52**, 19 (2003).
23. Q. Zhang, *J. Phys. D Appl. Phys.* **37**, 98 (2004).
24. P. C. Osbond, R. W. Whatmore, *Ferroelectrics* **118**, 93 (1991).
25. D. Fu, K. Suzuki, K. Kato, *Jpn. J. Appl. Phys.* **41**, L1103 (2002).
26. J. F. Nye, *Physical Properties of Crystals* (Oxford Univ. Press, 2003), chap. 10.
27. M. E. Lines, A. M. Glass, *Principles and Applications of Ferroelectrics and Related Materials* (Oxford Univ. Press, Oxford, 1977).

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Surface Self-Organization Caused by Dislocation Networks

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We report a new mechanism of self-organization that can lead to robust surface ordering. We have quantitatively analyzed the thermal motion of holes created by sulfur atoms in a silver monolayer on a ruthenium surface, which we observed in real time with scanning tunneling microscopy. We find that the stability of the array of holes is determined by the arrangement and structure of misfit dislocations in the film.

Solid surfaces and thin films often exhibit complex atomic structures at nanometer length scales. For example, thin films are often observed to "self-organize" into ordered patterns with unit cells containing tens to millions of atoms (*1*–*3*). These nanostructures span a large variety of physical systems, including boron nitride nanomeshes (*4*), hydrogen-bonded supramolecular assemblies (*5*), and stress-controlled etching of Ge films on Si (*6*). Various mechanisms have been proposed to explain the energetic driving forces for self-organization. For example, overlapping electric (*7*), magnetic (*8*), and bulk elastic strain fields (*9*, *10*) can all lead to self-organization on surfaces. However, many of these systems are too complex to measure these energies directly with sufficient accuracy and to allow detailed atomic models of the self-organization to be developed and tested.

We addressed this problem for a system with an atomic structure that can be precisely determined, and in doing so we identified a mechanism for self-organization in which misfit dislocations play a crucial role. In a previous study, Pohl *et al*. (*11*) found that when a monolayer (ML) of Ag grown on Ru(0001) is exposed to small amounts (<0.1 ML) of sulfur, which binds strongly enough to the substrate to displace Ag, a well-ordered triangular lattice of sulfur-filled vacancy islands ("holes") forms spontaneously. Room-temperature scanning tunneling microscopy (STM) observations revealed that these holes moved by thermal motion. We examined the correlations in the motion between neighboring holes to determine the relationship between the atomic structure of the film and the mechanism of interaction between the holes.

The nature of the forces between neighboring holes turns out to have a marked influence on the relative sizes of vibrations transverse and parallel to the line separating the holes. This vibrational anisotropy can be accurately reproduced with an atomic model in which the holes are connected by misfit dislocations (*12*), which separate regions of hexagonal close-packed (hcp) stacking and face-centered cubic (fcc) stacking. The stability of the hole array is governed by the energetic cost of distorting these dislocations. These distortions can be modeled with a two-dimensional (2D) Frenkel-Kontorova (FK) model (*13*) of the Ag film. In this model Ag-Ag interactions are accounted for by an effective spring constant, and the film-substrate interactions are parameterized by a sinusoidal corrugation.

Vibrations in the vacancy-island lattice can be observed in STM image sequences as reported by Pohl *et al*. (*11*). This motion can be seen in Fig. 1 in an overlay of two STM images acquired 12 s apart (*14*), and more directly in movies S1 and S2. We tracked the trajectories of each hole in extended STM measurements over several hours and recorded the separations $\mathbf{s}_{kl}$ between nearest-neighbor (NN) holes labeled k and l. The time-correlation function (Fig. 2B) probing the displacement of the holes from time t to time $t + \tau$ is $G(\tau) = \langle|\mathbf{s}_{kl}(t) - \mathbf{s}_{kl}(t + \tau)|^2\rangle$, where the average is over the pairs kl and times t. This function reveals the strong temperature dependence of the time scale of the vibrations, pointing to the thermal origin of the vibrations (*15*, *16*).

The histogram in Fig. 2C captures the excursions at room temperature of the NN hole-pair separations from their equilibrium values. The width w of this Gaussian distribution reflects the magnitude of the forces acting within the hole array. But to determine the origin of the hole-hole interaction, it is essential to examine the vibration anisotropy (see below). With the same data as in Fig. 2C, we separately measured the fluctuations along the line connecting the two NN holes (stretch mode, Fig. 2D) and perpendicular to this line (shear mode). Averaging over 450 pairs × 370 frames, we found a fluctuation amplitude of $\sigma_{\parallel}^2/d^2 = 1.81\ (\pm 0.05) \times 10^{-3}$ in the stretch direction and $\sigma_{\perp}^2/d^2 = 1.73\ (\pm 0.05) \times 10^{-3}$ in the shear direction, where d is the NN hole spacing.

To interpret w and to establish the relation between the ratio $\sigma_{\parallel}^2/\sigma_{\perp}^2$ and the force anisotropy, we used Monte Carlo (MC) simulations (*14*). First, we examined a simple pairwise interaction model where NN holes k and l interact through the spring (i.e., harmonic oscillator) potential $E_{lk} = E_{\mathrm{stretch}} = \frac{1}{2}K_{\parallel}(|\mathbf{s}_{kl}| - |\mathbf{s}^0_{kl}|)^2$ with the "spring constant" $K_{\parallel}$. Such a potential can account for interactions that generate spring-like central forces (*17*) such as, for example, the substrate-mediated elastic interactions, which have often been invoked to explain observations of self-organization (*18*–*21*).

In our MC simulations, we adjusted $K_{\parallel}$ to reproduce the overall magnitude of the measured vibrations given by the Gaussian width w in Fig. 2C. The simulation yields a ratio of $\sigma_{\parallel}^2/\sigma_{\perp}^2 = 0.70 \pm 0.01$, which differs significantly from the experimental value of $\sigma_{\parallel}^2/\sigma_{\perp}^2 = 1.05 \pm 0.06$ (Fig. 2D). Thus, the simple pairwise force model fails to describe our observations. To phenomenologically account for the measured vibrational anisotropy, we added an energy cost E_{shear} for rotating the hole-hole bond

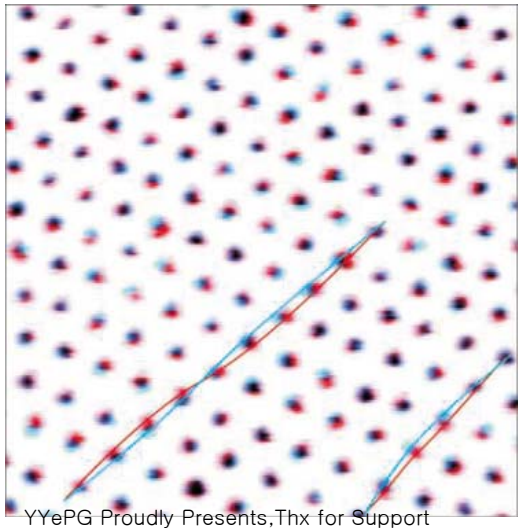

Fig. 1. Superposition of two STM images (red and cyan) acquired 12 s apart at T = 80°C, showing the thermal motion in the self-organized lattice of vacancy islands created by sulfur deposition onto a monolayer of Ag on Ru(0001). The average distance between the vacancy islands is 5.4 nm. The red and blue lines point to the presence of long-wavelength vibrations in the lattice. For the time dependence of this image, see movie S2.

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by an angle θ out of its equilibrium orientation (Fig. 2A):

$$E_{lk} = E_{\text{stretch}} + E_{\text{shear}} \quad (1)$$

with

$$E_{\text{stretch}} = \tfrac{1}{2}K_{\parallel}(|\mathbf{s}_{kl}| - |\mathbf{s}^0_{kl}|)^2 \quad (2)$$

and

$$E_{\text{shear}} = \tfrac{1}{2}K_{\perp}\, d^2\theta^2 \quad (3)$$

Adjusting $K_{\parallel}$ and $K_{\perp}$ to reproduce both measured vibration components, $\sigma^2_{\parallel}$ and $\sigma^2_{\perp}$, yields $K_{\parallel}$ = 3.2 eV/d^2 and a strong shear interaction of $K_{\perp}$ = 6.4 eV/d^2 (*22*, *23*).

To elucidate the origin of the unexpected strong shear forces, we performed simulations with an atomic 2D (FK) model (*13*, *14*). In this model, NN film atoms interact through harmonic pairwise forces and the substrate interaction is treated with a rigid sinusoidal 2D potential. The values we used for these interaction potentials are based on the experiments and density functional calculations reported by Thayer *et al*. (*24*).

Our model configuration (Fig. 3A) mimics the measured film structure (Fig. 3B). In particular, after relaxation, the model system accurately reproduces the misfit dislocations; as discussed in (*25*), these dislocations connect the neighboring holes and separate areas of hcp and fcc stacking (darker bands in Fig. 3, A, B, C, and E) (*26*–*28*). We can excite the vacancy lattice in a way that probes the interaction anisotropy by moving the lower row of vacancy islands and comparing the energy cost of horizontal shift versus vertical shift. The FK model system responds to these shifts (Fig. 3, C and E) by dragging the misfit dislocations along with the moving holes, ultimately distorting the dislocation network. The corresponding energy cost is plotted in Fig. 3, D and F. By evaluating these distortion energies (*29*), we can extract FK values for the stretch and shear interaction strengths of $K_{\parallel}$ = 3.5 eV/d^2 and $K_{\perp}$ = 5.0 eV/d^2. Considering the simplicity of the FK model, the agreement with the measured values $K_{\parallel}$ = 3.2 eV/d^2 and $K_{\perp}$ = 6.4 eV/d^2 is remarkable. Substrate-mediated elastic interactions, which had previously been proposed as the dominant ordering mechanism for the system studied here (*11*), would have a vanishing shear component $K_{\perp}$, which is very different from what we observe. In assuming a rigid substrate, the FK model by its nature excludes substrate-mediated interactions. Thus, reproducing the considerable shear interaction strength $K_{\perp}$ within the FK model establishes the dislocation network as the dominant source of the hole-hole interaction.

The success of the FK model enables us to investigate the role of the individual FK parameters in the stability of the hole array. We find that increasing the atomic spring constant or substrate corrugation increases the hole-array force constants. This dependence can be understood by considering the energy cost of distorting individual dislocations. For example, one might expect that the shear interaction is caused in part by a preference of the misfit dislocations to run along the directions where Ag and Ru atoms are closely packed. This preference gives rise to a stiffness $\tilde{\beta} = \beta(0) + d^2\beta(0)/d\theta^2$ of the misfit dislocation, where β(θ) is the angular dependence of the energy per unit length of the dislocation, with θ = 0 corresponding to the close-packed directions of the substrate.

The contributions of this stiffness to the energy cost of the distortions (Fig. 3, C and E) are easily shown to be $E_{\text{hori}} = 3\tilde{\beta}d\theta^2/2$ and $E_{\text{vert}} = \tilde{\beta}d\theta^2/2$. From the expressions for E_{hori} and E_{vert} given in (*29*), we find that the existence of the stiffness $\tilde{\beta}$ contributes a term $\tilde{\beta}/2d$ to $K_{\perp}$ but does not change $K_{\parallel}$. We directly computed the dislocation stiffness by distorting an isolated dislocation in our FK model and found that $\tilde{\beta} \approx 136$ meV/a, where a is the substrate lattice constant. Such distortion provides a considerable contribution of 1.4 eV/d^2 to $K_{\perp}$, and this contribution is long-ranged, decreasing as the square of the inverse hole separation. To relate the stiffness to atomic properties, we can use the results of Haldane and Villain (*30*), who showed that in the continuum limit of the FK model the stiffness is proportional to $\sqrt{kW}$, where k is the spring constant between Ag atoms and W depends on the substrate corrugation. Thus, the stronger the substrate corrugation and the stronger the in-plane spring constant, the more stable the hole array will be, as we calculate. Of course, calculating the distortion energy accurately requires the complete FK model calculation, because the dislocations interact and there are relaxations at the edges of the holes.

The mechanism that orders the array of S-filled holes embedded in an Ag monolayer on Ru(0001) requires only 2D elastic relaxations in the film, and thus it is quite different from the self-organization caused by bulk elastic relaxations found in other systems [e.g., Pb/Cu(111) (*19*) and O/Cu(110) (*18*)]. Unlike in ordered growth onto preexisting dislocation networks (*28*, *31*), the misfit dislocations mediating the ordering of this system emerge during the creation of the self-assembled lattice.

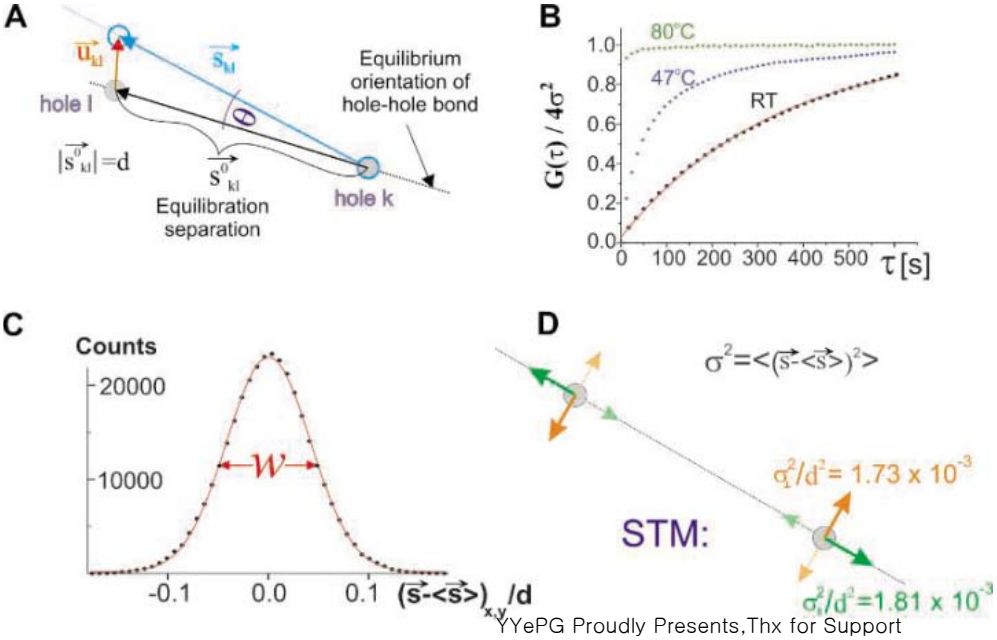

Fig. 2. Analysis of the lattice vibrations. (**A**) Schematic of a hole pair, using hole k as the reference point. (**B**) Time correlation function $G(\tau) = \langle|\mathbf{s}_{kl}(t) - \mathbf{s}_{kl}(t+\tau)|^2\rangle$ of the nearest neighbor (NN) hole-pair separation $\mathbf{s}_{kl}$ measured at various temperatures, normalized by the fluctuation $\sigma^2 = \langle(\mathbf{s}_{kl} - \langle\mathbf{s}_{kl}\rangle)^2\rangle$ of the NN hole-pair separation. (**C**) Histogram of the room-temperature fluctuations of NN hole separations. Both planar spatial components (x and y) of the fluctuations were sampled over 450 NN hole pairs and 370 images. (**D**) Anisotropy of the thermal hole vibrations at room temperature. The measured vibration amplitude along the connecting line of an NN hole pair (stretch mode) is slightly larger (5%) than the perpendicular shear component. In contrast, a stabilizing force acting only along the connecting line would result in a stretch amplitude smaller than the shear component by 30%.

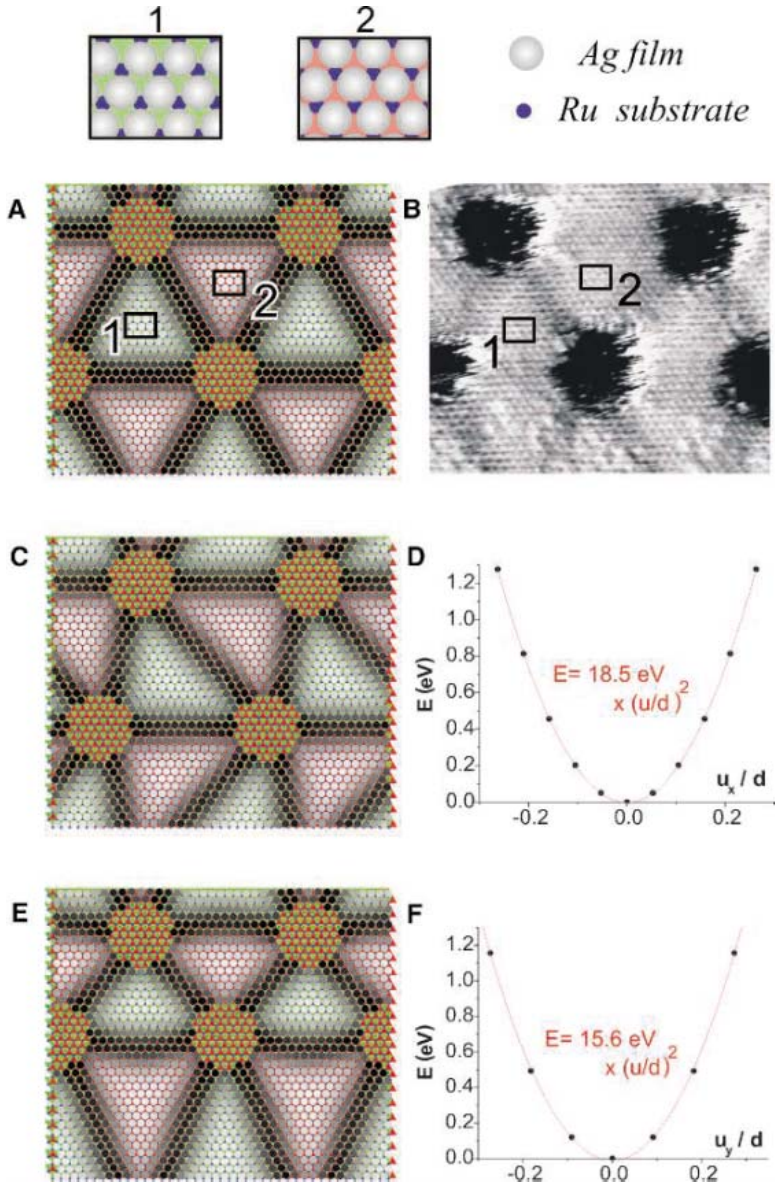

Fig. 3. FK simulation of the vacancy-island lattice. (**A**) The structure used in the FK model reproduces (**B**) the atomically resolved STM image (11 nm by 6 nm). Film atoms of the model system are represented by circles shaded according to the substrate potential at the respective site (black = high, white = low energy). The area between the circles is colored green or red to denote the type of stacking (fcc versus hcp). The insets 1 and 2 above (A) and (B) illustrate the stacking in adjacent regions separated by a partial dislocation. (**C** and **E**) Simulated response to shifting a row of holes out of their equilibrium positions. In (C) the lower row of holes is shifted horizontally, and in (E) vertically. (**D** and **F**) The corresponding energy cost as a function of the shift u measured in units of NN-hole spacing d.

Dislocations are ubiquitous in solids and are central to determining the structure and many properties of epitaxial thin films. Controlling the misfit in thin films by varying the composition of film or substrate offers the prospect of tailoring the spacing and pattern of self-organized lattices stabilized by the dislocations. For many of these systems, the FK model should be applicable and the values we obtained for our specific system could be used to estimate the interaction strength responsible for nanoscale order.

References and Notes

1. G. M. Whitesides, B. Grzybowski, *Science* **295**, 2418 (2002).
2. F. Rosei, *J. Phys. Condens. Matter* **16**, S1373 (2004).
3. J. V. Barth, G. Costantini, K. Kern, *Nature* **437**, 671 (2005).
4. M. Corso *et al.*, *Science* **303**, 217 (2004).
5. J. A. Theobald, N. S. Oxtoby, M. A. Phillips, N. R. Champness, P. H. Beton, *Nature* **424**, 1029 (2003).
6. F. Leroy, J. Eymery, P. Gentile, F. Fournel, *Surf. Sci.* **545**, 211 (2003).
7. D. Vanderbilt, *Surf. Sci.* **268**, L300 (1992).
8. Y. Yafet, E. M. Gyorgy, *Phys. Rev. B* **38**, 9145 (1988).
9. O. L. Alerhand, D. Vanderbilt, R. D. Meade, J. D. Joannopoulos, *Phys. Rev. Lett.* **61**, 1973 (1988).
10. V. I. Marchenko, *JETP Lett.* **33**, 381 (1981).
11. K. Pohl *et al.*, *Nature* **397**, 238 (1999).
12. Incorporating misfit dislocations at the interface between film and substrate is a common mechanism to relieve elastic strain due to the lattice mismatch between film and substrate. In the system studied here, this mismatch is considerable: The nearest neighbor distance of bulk Ag is 7% larger than that of Ru.
13. F. C. Frank, J. H. van der Merwe, *Proc. R. Soc. London Ser. A* **198**, 205 (1949).
14. See supporting material on *Science* Online.
15. The relaxation time is related to the mobility of the holes, which depends on the atomic diffusion mechanisms for their motion [see, for example, (*16*)].
16. S. V. Khare, N. C. Bartelt, T. L. Einstein, *Phys. Rev. Lett.* **75**, 2148 (1995).
17. P. Zeppenfeld, M. Krzyowski, C. Romainczyk, G. Comsa, M. G. Lagally, *Phys. Rev. Lett.* **72**, 2737 (1994).
18. G. Prevot *et al.*, *Surf. Sci.* **549**, 52 (2004).
19. R. van Gastel, R. Plass, N. C. Bartelt, G. L. Kellogg, *Phys. Rev. Lett.* **91**, 055503 (2003).
20. J. M. Rickman, D. J. Srolovitz, *Surf. Sci.* **284**, 211 (1993).
21. B. Croset *et al.*, *Phys. Rev. Lett.* **88**, 056103 (2002).
22. Besides contributing to the remarkably high degree of observed surface ordering, the large microscopic shear interaction $K_\perp$ also strongly affects the macroscopic elastic properties of the vacancy island crystal. For example, for a Cauchy solid (*23*) (one with only central microscopic interactions), the ratio between bulk modulus ($\sqrt{3}K_\parallel/2$) and shear modulus ($\sqrt{3}K_\parallel/4 + 3\sqrt{3}K_\perp/2$) is 2, but our results yield the much lower value of 0.15.
23. L. W. Bruch, M. W. Cole, E. Zaremba, *Physical Adsorption: Forces and Phenomena* (Clarendon, Oxford, 1997), p. 190.
24. G. E. Thayer *et al.*, *Phys. Rev. Lett.* **89**, 036101 (2002).
25. K. Thürmer, C. B. Carter, N. C. Bartelt, R. Q. Hwang, *Phys. Rev. Lett.* **92**, 106101 (2004).
26. The misfit dislocations run parallel to the surface, with their core located between the first and second atomic layers (*27*, *28*)—in this system, between the Ag monolayer and the Ru substrate. Their Burgers vectors are perpendicular to the dislocation lines and point from the hcp-stacking site to the fcc-stacking site, or vice versa, rendering them partial dislocations of pure edge character.
27. U. Harten, A. M. Lahee, J. P. Toennies, C. Wöll, *Phys. Rev. Lett.* **54**, 2619 (1985).
28. D. D. Chambliss, R. J. Wilson, S. Chiang, *J. Vac. Sci. Technol.* **B9**, 933 (1991).
29. Quadratic fits of the distortion energy $E(u)$ yield $E_{hori} = 18.5\ eV \times (u/d)^2$ and $E_{vert} = 15.6\ eV \times (u/d)^2$. Assuming only small shifts u, simple geometrical considerations yield the distortion energies for horizontal shifts $E_{hori}^{stretch} = K_\parallel u^2$ and $E_{hori}^{shear} = 3K_\perp u^2$ and for vertical shifts $E_{vert}^{stretch} = 3K_\parallel u^2$ and $E_{vert}^{shear} = K_\perp u^2$. Adding stretch and shear energy yields the total energies $E_{hori} = (K_\parallel + 3K_\perp) \times u^2$ and $E_{vert} = (3K_\parallel + K_\perp) \times u^2$. Solving these equations for $K_\parallel$ and $K_\perp$, we can extract FK values for the stretch and shear interaction strengths of $K_\parallel = 3.5\ eV/d^2$ and $K_\perp = 5.0\ eV/d^2$.
30. F. D. M. Haldane, J. Villain, *J. Phys. (Paris)* **42**, 1673 (1981).
31. H. Brune, M. Giovannini, K. Bromann, K. Kern, *Nature* **394**, 451 (1998).

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Fig. S1
Movies S1 and S2

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Synthesis and Characterization of the Nitrides of Platinum and Iridium

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Transition metal nitrides are of great technological and fundamental importance because of their strength and durability and because of their useful optical, electronic, and magnetic properties. We have evaluated a recently synthesized platinum nitride (PtN) that was shown to have a large bulk modulus, and we propose a structure that is isostructural with pyrite and has the stoichiometry PtN_2. We have also synthesized a recoverable nitride of iridium under nearly the same conditions of pressure and temperature as PtN_2. Although it has the same stoichiometry, it exhibits much lower structural symmetry. Preliminary results suggest that the bulk modulus of this material is also very large.

The importance of transition metal nitrides is well known (*1–5*). Recently, several experimental investigations have been made into the synthesis and properties of nitrides produced under extreme conditions of pressure and temperature. Novel phases of known nitrides have been successfully synthesized (*6*, *7*), as well as a bulk nitride of the noble metal platinum (*1*). The latter material was found to be stable under ambient conditions and to possess a bulk modulus of 372 GPa, notable for being much larger (by nearly 100 GPa) than the pure metal, contrary to the usual behavior of transition metal nitrides. These investigations suggest intriguing possibilities for the synthesis under extreme conditions of other nitrides with notable or distinctive properties.

Several theoretical investigations of platinum nitride have now also been carried out (*8–15*), and the consensus appears to be that the compound does not crystallize in the proposed zinc-blende structure (*1*), because this arrangement would violate the requirement of positive strain energy (*12–15*). Yu and Zhang (*12*, *15*) have furthermore suggested that platinum nitride is instead stable in the fluorite structure, an arrangement in which the nitrogen atoms occupy all the tetrahedral interstitial sites of the face-centered cubic (fcc) metal lattice and which necessitates a stoichiometry of PtN_2. They found the corresponding bulk modulus (290 GPa) to be larger than that of pure platinum (276 GPa), but nevertheless much smaller than the experimental value (372 GPa) (*1*).

We initially considered the thermodynamic stability of the proposed zinc-blende and fluorite structures as a function of pressure. This was done by performing density functional calculations of the Pt-N system within the generalized gradient approximation (GGA) in the PW91 parametrization (*16*). We used the Vienna ab initio simulation package that implements the projector augmented wave (PAW) method (*17*,*18*). Having reproduced the previously calculated equilibrium lattice constants and the bulk moduli of the zinc-blende and the fluorite structures (*12*), we calculated their formation energies at ambient pressure as well as at 50 GPa. The latter were obtained by subtracting the chemical potentials of the constituents in their pure phases from that of the proposed structure. The chemical potential of N_2 was determined using its experimental equation of state (*19*). Hence, we correct for the density functional theory–GGA (DFT-GGA) overbinding of the nitrogen dimer; an error of about 0.7 eV/(N_2). Our calculations show that both structures are highly unstable at both ambient pressure and 50 GPa. The calculated formation energies of the zinc-blende structure at a pressure of 0 GPa and 50 GPa are found to be 1.9 eV/(PtN structural unit) and 2.2 eV/(PtN), whereas those of the fluorite structure are 3.5 eV/(PtN_2) and 2.1 eV/(PtN_2), respectively. The zinc-blende structure becomes less stable under pressure because this structure is less dense than the constituents in their pure phases.

In light of these results, further experimental characterization was required. We synthesized the nitride using a technique similar to that described in (*1*), that is, by laser heating the relevant materials under high pressure in a diamond anvil cell (DAC). We did the same for iridium for comparison with Pt-N. The relevant metal was placed into the DAC cavity either in the form of thin squares (~30 by 10 μ) or fine powder. Nitrogen was then loaded into the DAC cryogenically. Raman spectroscopy was used to determine when a reaction had occurred. [See (*20*) for further details.]

In the pressure range of interest, the only features of the Raman spectra were due to nitrogen (specifically the ε phase). Moreover, those in the relevant frequency range (100 to 1200 cm^{-1}) were comparatively weak and broad (nitrogen lattice modes).

For both metals, compound formation was indicated by the sudden appearance after heating of a number of intense and well-defined modes (Fig. 1). In the case of platinum, we found synthesis conditions in close agreement with (*1*), that is, ~50 GPa and 2000 K. For iridium nitride, we found that synthesis did not occur below 47 GPa and 1600 K (*21*).

The measured Raman spectrum after reaction with platinum exhibited two intense modes at around 860 cm^{-1} and 1020 cm^{-1}, with two weaker modes at 790 cm^{-1} and 1050 cm^{-1} (see Fig. 1A, which displays spectra acquired at 0 GPa and 50 GPa). This was in marked contrast to that of iridium, which contained at least 11 modes (Fig. 1B) (*22*).

This observation and all details of the spectra were reproducible and independent of the initial form of the metal. The frequencies of the observed modes after reaction with iridium are displayed as a function of pressure in Fig. 1C. On the basis of the Raman modes and their pressure dependence, we concluded that the products of the reaction are structurally stable and highly crystalline to at least 80 GPa.

Group-theory arguments show that there is only one Raman-active mode for the zinc-blende structure, which in the case of ionically bonded solids may be polar. It can thus be split into two components, transverse and longitudinal. Furthermore, the fluorite structure proposed by Yu and Zhang (*12*, *15*) should possess only a single active mode (*15*). By contrast, we observe four modes in Pt-N. This indicates a lower symmetry than either the zinc-blende or fluorite structures. In fact, the form of our spectrum matches closely that of pyrite (FeS_2) (*23–26*). In the case of iridium nitride, the rich structure of the Raman spectrum suggests either more atoms in the unit cell and/or a less symmetric structure.

Scanning electron microscopy of the recovered nitrides revealed regions that had a pronounced surface texture (fig. S1). Bulk-sensitive energy-dispersive x-ray spectroscopy (EDX) was used to demonstrate the complete correlation between these regions and the presence of nitrogen. Using EDX, we found variable stoichiometries for both PtN_x and IrN_x, with x ranging from 0.6 to >2. Because unreacted metal was present in the recovered products and the characteristic penetration depth of the technique was some hundreds of nanometers, it is not surprising that values for $x < 1$ were observed. The possibility of sub- or super-stoichiometric phases containing nitrogen or metal vacancies, respectively, should also be considered. To obtain more precise information, we employed surface-sensitive x-ray photoelectron spectroscopy (XPS). As opposed to EDX, XPS provides not only atomic composition but also information on chemical bonding. Incontrovertible evidence of the formation of compounds of iridium and platinum was obtained in the form of a binding energy shift of the 4f spin-orbit pair compared with that of the elemental metal. Specifically, the Pt $4f_{7/2}$ binding energy for PtN_x is 74.0 eV versus 70.8 eV for our Pt metal standard. The Ir $4f_{7/2}$ binding energy for IrN is 61.8 eV versus 60.7 eV for elemental Ir. Nitrogen binding energies (N1s) similarly displayed a shifted component indicative of compound formation. In the case of platinum the energy of the shifted N1s peak (399.0 eV) was in good agreement with that measured by Soto (*27*), who synthesized PtN_x thin films. This energy was somewhat larger than the typical value for other transition metal nitrides, for example, hafnium nitride (*28*), and suggests some covalent bonding.

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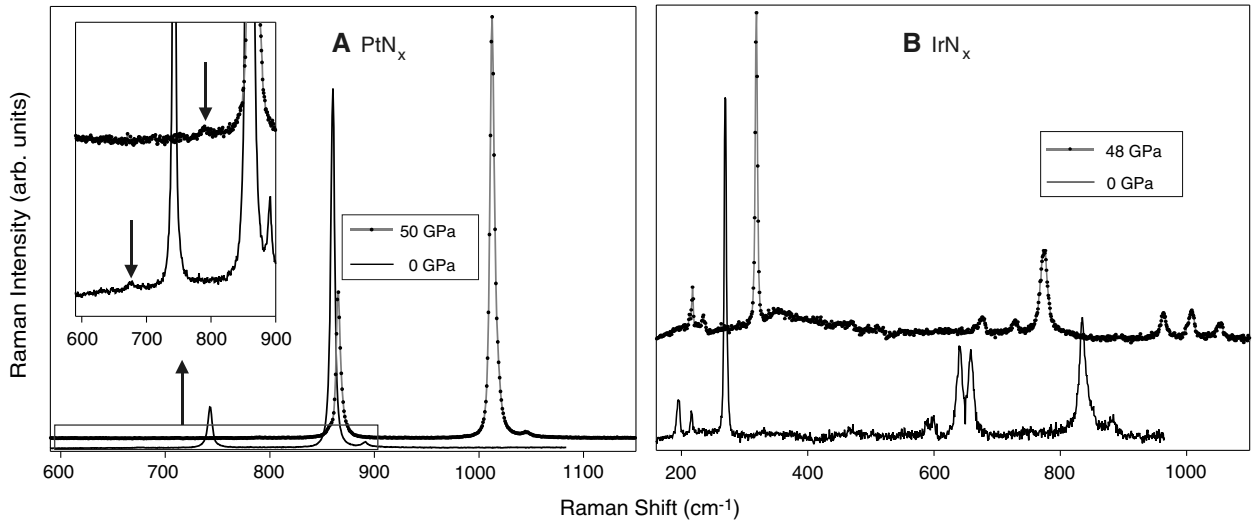
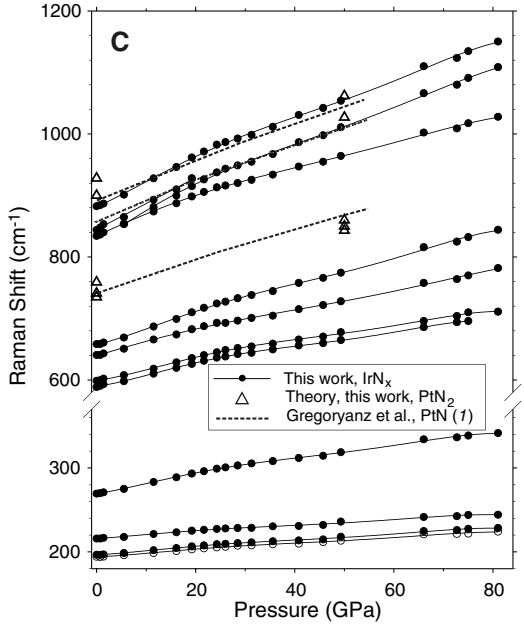


Fig. 1. (**A**) Raman spectra of platinum nitride at ambient pressure and at 50 GPa (nitrogen lattice modes have been subtracted from the latter spectrum). The arrow in the inset indicates a weak but consistently observed peak. Modes are assigned assuming a pyrite structure (from lowest to highest frequency): T_g, E_g, A_g, T_g (*23*–*26*). (**B**) Raman spectra after heating iridium in the presence of nitrogen at 48 GPa and of the recovered product at 0 GPa. (**C**) Dependences of observed Raman modes of iridium nitride on pressure. The lines are second-order polynomials. The corresponding derivatives at ambient pressure are (in order of descending ambient pressure mode frequency): 4.72, 4.03, 3.77, 3.06, 2.45, 2.25, 2.29, 1.48, 0.59, 0.63, and 0.57. Also shown are the dependences for platinum nitride as reported in (*1*).



By comparing only the shifted components of each element together with the appropriate sensitivity factors, we obtained for x a value of 2 ± 0.5 for both platinum nitride and iridium nitride (*29*).

On this basis, we explored from first principles the behavior of the Pt-N system, assuming the pyrite structure. The latter is cubic with 12 atoms per primitive unit cell and consists of four Pt atoms arranged in fcc positions and four pairs of N atoms aligned along one of the (111) directions. The midpoints of the nitrogen pairs are also arranged on an fcc lattice, which together with the Pt atoms results in a NaCl-type arrangement (Fig. 2A). The pyrite structure has one free parameter (u) that determines the bond length between the nitrogen pairs. The fluorite structure is a particular high-symmetry phase of pyrite having $u = 0.25$, which leads to an N-N bond length of 4.16 Å for the equilibrium lattice constant (a_{lat}) of 4.8 Å.

Figure 2B shows the PAW-GGA prediction of the formation energy of PtN_2 in the pyrite structure as a function of u at the GGA equilibrium lattice constant (4.87 Å). It confirms that the fluorite structure (u = 0.25) is at a local minimum; however, the structure with $u = 0.415$ is lower in energy by almost 2.7 eV per structural unit (i.e., PtN_2). This is a dramatic difference in energy and is confirmed by full-potential linear-augmented plane-wave calculations using the WIEN2k code (*30*). We nevertheless found that this structure has a positive energy of formation of about 0.72 eV per structural unit at ambient pressure. However, at P = 50 GPa, the pyrite phase becomes thermodynamically stable, with a formation energy of about –1.2 eV/(PtN_2), in good agreement with the experimental observations.

We calculated the frequencies of all the Raman-active modes within the GGA for the two lattice constants: 4.80 Å and 4.62 Å, corresponding to the experiments at ambient pressure and 50 GPa, respectively (Table 1). The pyrite structure has five Raman-active modes, one A_g, one E_g, and three T_g modes (*26*). The degeneracy is one, two, and three for the A_g, E_g, and T_g modes, respectively. Experimentally, we observed four of the five modes with all calculated frequencies within 10% or better of the experimental values (*31*). Dependences on pressure are also well reproduced (Fig. 1C).

Previous theoretical calculations of PtN_2 in the fluorite structure predicted a lattice constant within the local density approximation (GGA) of 4.866 (4.958) Å, whereas the pyrite structure is at equilibrium for a_{lat} = 4.79 (4.875) Å, in better agreement with the experimental value of 4.8 Å. Furthermore the bulk modulus for the new structure of 347 (278) GPa is also significantly higher than the previously calculated values of 316 (264) GPa for the fluorite structure, consistent with (*1*), that is, that the bulk modulus of platinum nitride is larger than that of platinum.

The atoms in the nitrogen pairs of our equilibrium pyrite structure are much more tightly bound, with a bond length of only 1.41 Å, than in the fluorite structure. It is intriguing that this

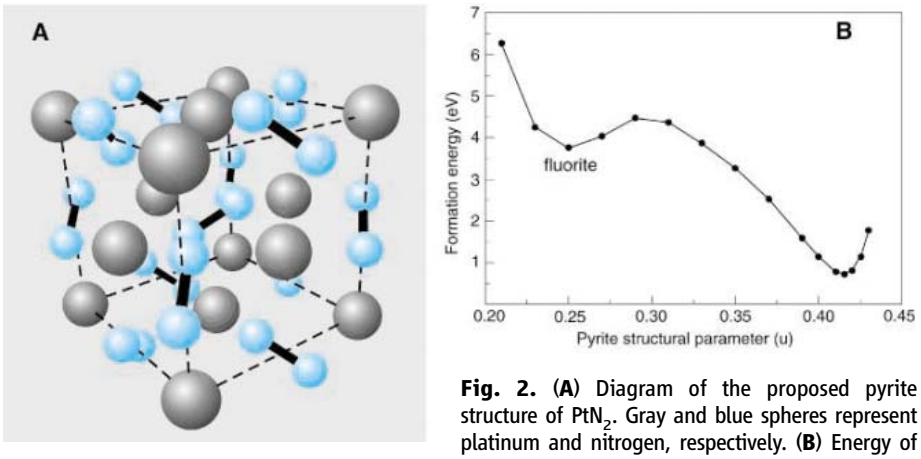


Fig. 2. (**A**) Diagram of the proposed pyrite structure of PtN_2. Gray and blue spheres represent platinum and nitrogen, respectively. (**B**) Energy of formation of the pyrite phase of PtN_2 versus structural parameter *u*.

Table 1. The frequencies of all Raman-active modes in pyrite PtN_2 calculated within the PAW-GGA for the two lattice constants a_{lat} = 4.80 Å and 4.62 Å, corresponding to the pressures 0 GPa and 50 GPa, respectively. Corresponding experimental frequencies are shown in parentheses.

	$\nu(T_g)$ (cm^{-1})	$\nu(E_g)$ (cm^{-1})	$\nu(T_g)$ (cm^{-1})	$\nu(A_g)$ (cm^{-1})	$\nu(T_g)$ (cm^{-1})
P = 0 GPa	735 (676)	741 (743)	759 (N/O)	900 (860)	928 (891)
P = 50 GPa	843 (791)	850 (865)	860 (N/O)	1027 (1013)	1062 (1046)

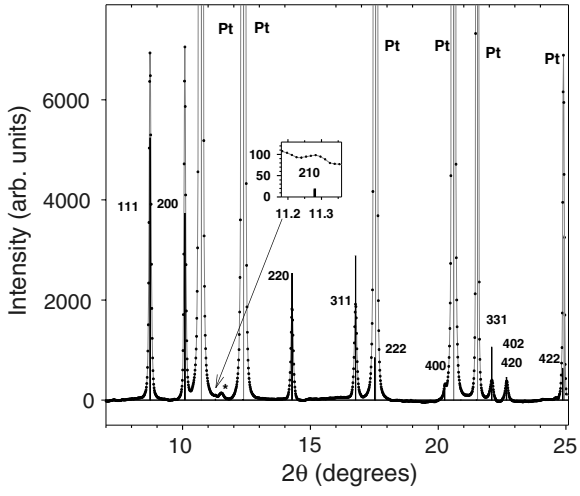


Fig. 3. Powder x-ray diffraction pattern of PtN_2 obtained at 0 GPa. The reflections of PtN_2 are indexed assuming the pyrite structure. Black vertical bars show the calculated positions and intensities of the reflections. The gray vertical lines indicate unreacted platinum. The star corresponds to the 101 reflection of rhenium. The inset shows a very weak reflection that is forbidden for the more symmetric fluorite and zinc-blende structures.

separation is almost exactly the same as for the single-bonded monatomic (triply coordinated) nitrogen that has been predicted theoretically (*32*) and that has recently been reported in an experimental study (*33*). The nitrogen bonding in this structure is purely covalent. It can also be shown that all the first-order Raman modes in the pyrite structure of PtN_2 correspond to vibrations of nitrogen. It is the N-N bond that accounts for the high Raman frequencies (in particular the A_g mode at 860 cm^{-1}, due to symmetric N-N stretching) and the large Raman scattering cross-section (*1*). These characteristics of the bonding are likely responsible for the large bulk modulus.

We have also made preliminary powder x-ray diffraction (XRD) measurements on both iridium and platinum nitride as a function of pressure. The diffraction pattern of PtN_2 is dominated by the fcc metal sublattice and is consistent with the proposed pyrite structure (Fig. 3). By contrast, the pattern of IrN_2 can only be reconciled with a structure of much lower symmetry than cubic. Equation-of-state measurements were performed during the release of pressure after the synthesis of the compounds. A broadening of the Bragg reflections and a relatively large scatter in the acquired data limited the precision with which the equation of state could be acquired. These problems were most likely a consequence of deviatoric stresses (Fig. 3), because the pressure medium lacked complete hydrostaticity and the sample was grown on and remained in contact with the metal substrate. Nevertheless, the change of the d-spacings of both materials with pressure is consistent with large bulk moduli in the range of 350 to 410 GPa [consistent with (*1*)] (*34*).

References and Notes

1. E. Gregoryanz *et al.*, *Nat. Mater.* **3**, 294 (2004).
2. S. T. Oyama, *The Chemistry of Transition Metal Carbides and Nitrides*, S. T. Oyama, Ed. (Blackie Academic and Professional, London, 1996), chap. 1.
3. H. A. Pierson, *Handbook of Refractory Carbides and Nitrides* (Noyes, Westwood, NJ, 1996), chaps. 9 to 13.
4. S. H. Jhi, J. Ihm, S. G. Louie, M. L. Cohen, *Nature* **399**, 132 (1999).
5. P. Kroll, *Phys. Rev. Lett.* **90**, 125501 (2003).
6. A. Zerr, G. Miehe, R. Boehler, *Nature* **400**, 340 (1999).
7. A. Zerr, G. Miehe, R. Boehler, *Nat. Mater.* **2**, 185 (2003).
8. B. Sahu, L. Kleinman, *Phys. Rev. B* **71**, 041101 (2005).
9. B. Sahu, L. Kleinman, *Phys. Rev. B* **72**, 119901 (2005). [erratum].
10. J. Uddin, G. E. Scuseria, *Phys. Rev. B* **72**, 035101 (2005).
11. J. Uddin, G. E. Scuseria, *Phys. Rev. B* **72**, 119902 (2005). [erratum].
12. R. Yu, X. F. Zhang, *Appl. Phys. Lett.* **86**, 121913 (2005).
13. S. K. R. Patil, S. V. Khare, B. R. Tuttle, J. K. Bording, S. Kodambaka, *Phys. Rev. B*, in press.
14. C.-Z. Fan *et al.*, *Chin. Phys. Lett.* **22**, 2637 (2005).
15. R. Yu, X. F. Zhang, *Phys. Rev. B* **72**, 054103 (2005).
16. J. P. Perdew, Y. Wang, *Phys. Rev. B* **45**, 13244 (1992).
17. G. Kresse, J. Furthmuller, *Phys. Rev. B* **54**, 11169 (1996).
18. G. Kresse, D. Joubert, *Phys. Rev. B* **59**, 1758 (1999).
19. H. J. Olijnyk, *Chem. Phys.* **93**, 8968 (1990).
20. Materials and methods are available as supporting material on *Science* Online.
21. Pressure was measured before and after heating and was found not to vary within the measurement precision. Thermal pressure probably implies a synthesis pressure 1 to 2 GPa higher than that measured.
22. We have reported preliminary data for IrN_x in the form of a Raman spectrum (*35*).
23. H. Vogt, T. Chattopadhyay, H. J. Stolz, *J. Phys. Chem. Solids* **44**, 869 (1983).
24. T. P. Mernagh, A. G. Trudu, *Chem. Geol.* **103**, 113 (1993).
25. A. K. Kleppe, A. P. Jephcoat, *Mineral. Mag.* **68**, 433 (2004).
26. M. Blanchard *et al.*, *J. Phys. Chem. B* **109**, 22067 (2005).
27. G. Soto, *Mater. Lett.* **58**, 2178 (2004).
28. A. Arranz, *Surf. Sci.* **563**, 1 (2004).
29. Because we could not prevent the appearance of carbon in the EDX or XPS spectra of the recovered product, regardless of the form of the metal or how it was cleaned, the possibility arises that the large concentration of nitrogen may be due to the formation of one or several different carbon nitride compounds. (The source of the carbon may have been the diamonds themselves. In an attempt to suppress this possible migration of carbon, we also performed an experiment in which the diamonds were isolated from the sample materials by thin alumina plates. The observed Raman spectra after heating were indistinguishable from those of the other experiments.) However, comparison of measured Raman spectra with theoretical calculations of those of crystalline carbon/nitrogen compounds yielded no obvious correlation (*36*). We also did not observe any feature in the Raman spectra that corresponded to an amorphous carbon nitride. Moreover, the Raman spectra of both nitrides contained no common features. Oxygen was also observed in the EDX and XPS spectra (presumably adsorbed from the atmosphere in the elemental form or as a component of water). Because oxides of both platinum and iridium are known to exist, this possibility ought to be examined. We note, however, that for both iridium and platinum, the 4f peaks could be well fitted with two sets of doublets (one being the elemental), suggesting the presence of only one compound. In light of the Raman spectra and the known presence of nitrogen, together with the fact that the XPS spectra were acquired over the whole extent of the sample (or at least over hundreds of square microns of its extent), it seems unlikely that the compound in question was an oxide. Furthermore, the observed energies of the oxygen 1s peaks did not match those reported for the metal oxides [see (*37*), for example, which presents XPS results for IrO_2] but were consistent with those of adsorbed water (*38*).
30. P. Blaha, K. Schwarz, J. Luitz, *Computer Code WIEN2k*, www.wien2k.at, Techn. Univ. Wien, Vienna (2001).
31. In the unpolarized Raman scattering measurements of (*25*) on FeS_2 pyrite, only four modes were resolved.

The missing mode was also a T_g mode and was obscured by the more intense A_g mode. In the polarized measurements of (*23*), all three T_g modes were observed, but only in specific polarization configurations. The same was true for the other compounds with the pyrite structure that were studied in (*23*). We therefore believe that the most likely explanation for the missing T_g mode in our spectra is its intrinsic weakness and proximity to either the intense E_g or A_g mode.
32. C. Mailhiot, L. H. Yang, A. K. McMahan, *Phys. Rev. B* **46**, 14419 (1992).
33. M. I. Eremets, A. G. Gavriliuk, I. A. Trojan, D. A. Dzivenko, R. Boehler, *Nat. Mater.* **3**, 558 (2004).
34. In the case of IrN_2, the pressure dependences of the different d-spacings were the same within the experimental uncertainty, and the bulk modulus was thus obtained assuming isotropic compressibility. The corresponding large uncertainties in the moduli are related to the scatter in the data and also to an unconstrained pressure derivative of the bulk modulus.
35. A. F. Goncharov, J. C. Crowhurst, *J. Low Temp. Phys.* **139**, 727 (2005).
36. G.-M. Rignanese, J.-C. Charlier, X. Gonze, *Phys. Rev. B* **66**, 205416 (2002).
37. M. J. Bozack, *Surf. Sci. Spectra* **2**, 123 (1994).
38. D. Barreca, G. A. Battiston, R. Gerbasi, E. Tondello, P. Zanella, *Surf. Sci. Spectra* **7**, 303 (2000).
39. We acknowledge several illuminating discussions with T. Barbee, R. Yu, and T. Lenosky. The assistance of Y. Meng in performing XRD measurements, M. Somayazulu in interpreting XRD spectra, and L. Zepeda-Ruiz in preparing Fig. 2A was much appreciated. We thank the three anonymous reviewers for their comments. Use of the Advanced Photon Source was supported by the U.S. Department of Energy (DOE), Office of Science, Office of Basic Energy Sciences, under contract W-31-109-ENG-38. Use of the High Pressure Collaborative Access Team facility was supported by DOE Basic Energy Sciences, DOE National Nuclear Security Administration, NSF, Department of Defense Tank-Automotive and Armaments Command, and the W. M. Keck Foundation. This work was preformed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory (LLNL), under contract W-7405-ENG-48 and was supported by the Laboratory Directed Research and Development office of the LLNL..

Supporting Online Material

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Materials and Methods
Fig. S1
References

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The Rotational Spectrum of the Water–Hydroperoxy Radical (H_2O–HO_2) Complex

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Peroxy radicals and their derivatives are elusive but important intermediates in a wide range of oxidation processes. We observed pure rotational transitions of the water–hydroperoxy radical complex, H_2O–HO_2, in a supersonic jet by means of a Fourier transform microwave spectrometer combined with a double-resonance technique. The observed rotational transitions were found to split into two components because of the internal rotation of the water moiety. The molecular constants for the two components were determined precisely, supporting a molecular structure in which HO_2 acts as a proton donor to form a nearly planar five-membered ring, and one hydrogen atom of water sticks out from the ring plane. The structure and the spectral splittings due to internal rotation provide information on the nature of the bonding interaction between open- and closed-shell species, and they also provide accurate transition frequencies that are applicable to remote sensing of this complex, which may elucidate its potential roles in atmospheric and combustion chemistry.

The hydroperoxyl radical (HO_2) is implicated as a transient intermediate in a wide range of oxidation processes. Recently, the potential importance of its water complex has attracted much attention in combustion and atmospheric reaction models (*1*–*8*). For example, self-reaction of HO_2 is a primary source of atmospheric hydrogen peroxide (H_2O_2)

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \qquad (1)$$

It was found that water enhances the rate of reaction (1) more efficiently than expected by considering only the effect of collisional energy transfer between HO_2 and H_2O (*1*). Hamilton and Lii ascribed this effect to the formation of the H_2O–HO_2 complex, because the water complex reacts faster than the isolated HO_2 does (*2*). Furthermore, H_2O–HO_2 is estimated to have a fairly large binding energy, 9.4 kcal/mol (*3*), and it has many low-frequency intermolecular vibrational modes. The equilibrium constant for the formation of the water complex

$$H_2O + HO_2 \rightleftharpoons H_2O{-}HO_2 \qquad (2)$$

is thus expected to be relatively large. Because of the abundance of atmospheric water, a certain amount of H_2O–HO_2 is posited to exist in the atmosphere, where it could act as a sink of the atmospheric HO_2 radical. Approximately 20 to 30% of HO_2 in the atmosphere is estimated to form complexes with H_2O under certain conditions (*3*, *4*). Many experimental (*4*–*6*) and theoretical studies (*3*, *7*, *8*) have been performed to characterize this complex and to understand the mechanism of the enhancement of the recombination reaction. However, many of these studies are still far from conclusive, mainly because there are no experimental studies that directly detect H_2O–HO_2 in the gas phase.

High-resolution spectroscopic studies can provide precise information on the geometries and large-amplitude internal motions of such weakly bound complexes. These studies in turn can be used to estimate reaction parameters such as the equilibrium constant of eq. 2. Spectroscopic studies also yield accurate transition frequencies for use in direct atmospheric detection.

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The H_2O–HO_2 complex is interesting for fundamental bonding insights as well. Although a large number of studies have been reported for complexes consisting of closed-shell monomers (*9*), only a few high-resolution spectroscopic studies have been reported for closed-shell molecule–open-shell radical complexes in the gas phase. Some of the complexes studied contain stable open-shell monomers, such as NO–HF (*10*) and H_2O–O_2 (*11*), whereas others contain a reactive monomer, such as OH–M [M = H_2O (*12*, *13*) or CO (*14*)]. The role of the unpaired electron in such complexes is a continuing area of study.

We observed pure rotational transitions of the H_2O–HO_2 radical complex with Fourier transform microwave (FTMW) spectroscopy as well as the double-resonance technique recently introduced in our laboratory (*15*, *16*). In the double-resonance technique, higher frequency mm-wave radiation was sent into the Fabry-Perot cavity of the FTMW spectrometer, and induced transitions beyond 40 GHz were observed as a change in intensity of a lower frequency resonance. Before the spectral search, we performed ab initio calculations to estimate the frequency regions in which transitions were likely to appear. The most stable structure predicted with restricted coupled-cluster methods [RCCSD(T)/aug-cc-pVTZ] is a five-membered ring (Fig. 1) (*17*, *18*). A two-dimensional potential energy surface of H_2O–HO_2 about the two internal rotation angles of the H_2O moiety is shown in Fig. 2. Four minima in the figure are connected by the symmetry operations of the group G_4 {E, (12), E*, (12)*} (*19*, *20*), where the symmetry operations (12), E*, and (12)* are the permutation of the two hydrogen nuclei of the H_2O moiety, the inversion, and the simultaneous operations of (12) and E* [(12) × E* = (12)*], respectively. Because the barrier heights for the E* and (12)* operations are approximately 200 cm^{-1} (Table 1), all the symmetry operations of the group G_4 are expected to be feasible, causing tunneling splittings in the pure rotational spectra.

The HO_2 radical was produced by an electric discharge (1.5 kV) in a gas mixture of O_2 (10%) and Ar, passed through a reservoir filled with pure water within a pulsed discharge nozzle (*21*, *22*), and was subsequently cooled to form the complex in a supersonic expansion with a stagnation

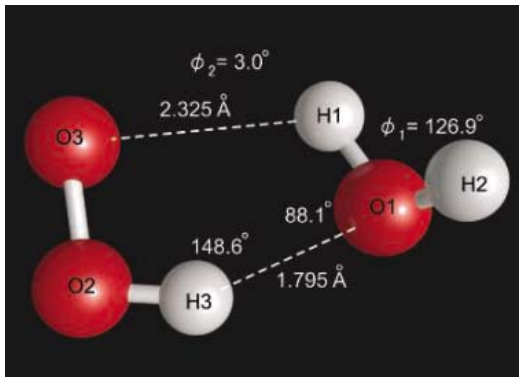

Fig. 1. The molecular structure of the H_2O–HO_2 complex calculated at the RCCSD(T)/aug-cc-pVTZ level of theory. Intermolecular structural parameters are shown in the figure. The structural parameters of the monomers are $r_{H_1O_1}$ = 0.967 Å, $r_{H_2O_1}$ = 0.962 Å, $\angle(H_1O_1H_2)$ = 105.2°, $r_{H_3O_2}$ = 0.987 Å, $r_{O_2O_3}$ = 1.334 Å, and $\angle(H_3O_2O_3)$ = 102.6°.

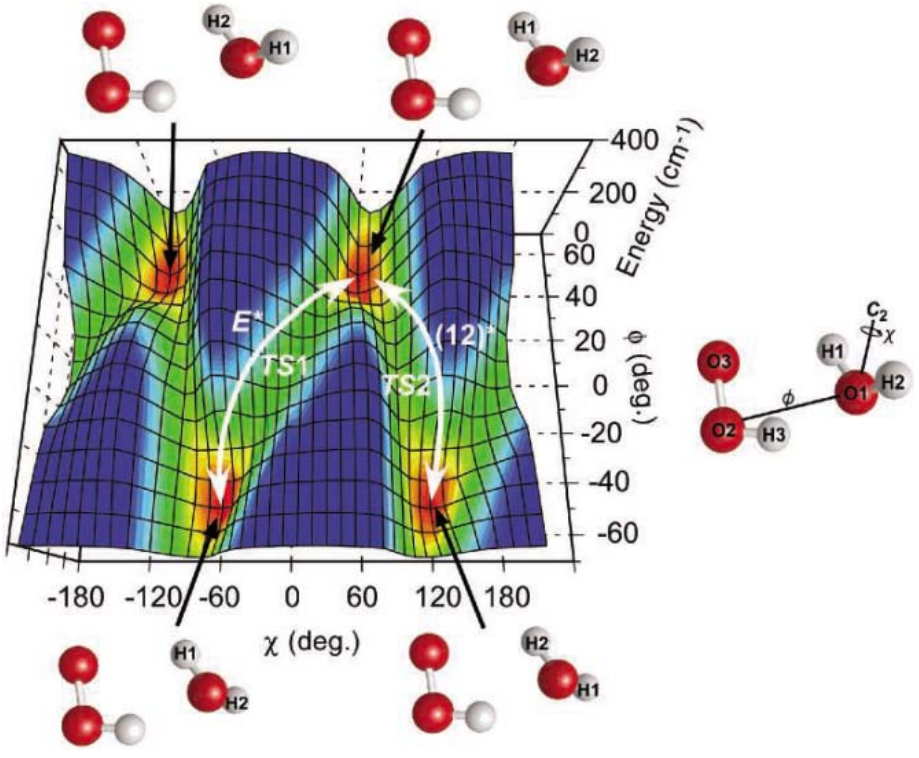

Fig. 2. Two-dimensional potential energy surface of H_2O–HO_2 obtained at the B3LYP/aug-cc-pVDZ level of theory. Molecular parameters, except for φ and χ, were optimized at each point, whereas the monomer structure was fixed. Definitions of the angles φ and χ are also shown, where the dihedral angle φ describes the orientation of the water C_2 axis with respect to the HO_2 plane, and χ describes the rotation angle of the molecular plane of the water around the C_2 axis.

Table 1. Comparison of the rotational constants *A*, *B*, and *C* obtained by theoretical calculations with the experimental values. GM stands for the global minimum, and TS1 and TS2 stand for the transition states (TS1) and (TS2) shown in the potential energy surface in Fig. 2. The theoretical rotational constants and energies were calculated at the RCCSD(T)/aug-cc-pVTZ level of theory. exp., experiment.

	GM	TS1	TS2	exp.
A (MHz)	31806	32552	34064	32897
B (MHz)	5968	5875	5074	5656
C (MHz)	5077	4976	4509	4829
ΔE (cm^{-1})*	0	215	206	–

*ΔE values are the energies of the transition states relative to the global minimum.

pressure of 5 atm. Three successive *a*-type *R*-branch transitions with N'' = 0, 1, and 2 for the K_a = 0 manifold, with large spin doublings and small, rather-complicated hyperfine splittings, were observed by FTMW spectroscopy in the 10- to 31-GHz region. Here, *N* is the rotational angular momentum, K_a is its projection on the molecular axis, and the double prime indicates the quantum number *N* for the lower state. Weak satellite lines were also observed around the region for $N = 2 \leftarrow 1$, corresponding to the transitions for the K_a = 1 manifold. The K_a = 0 transitions with $N = 4 \leftarrow 3$ at 41 GHz were observed by the double-resonance technique, monitoring the transitions with $N = 3 \leftarrow 2$ (*15*, *16*). A typical observed FTMW spectrum is shown in Fig. 3. The observed transitions could be divided into two groups by checking the connectivity of the transitions with the double-resonance technique. These two groups of lines have different hyperfine patterns, whereas other features, such as the production conditions and the paramagnetic behaviors, are quite similar. One group of lines shows the features of a radical with two nuclear spins, I_1 = ½ and I_2 = 1, with larger (~MHz) and smaller (~100 kHz) splittings. The other group of lines has only one nuclear spin, I_1 = ½ (namely I_2 = 0). We concluded that the permutation (12) is feasible for this complex, and we assigned these two groups of lines to the pure rotational transitions belonging to two different vibrational states of the intermolecular motions.

Because hydrogen nuclei obey the Fermi-Dirac statistics, the overall wave functions must be antisymmetric with respect to the permutation (12). Therefore, ortho states (I_2 = 1) are assigned to the spatially antisymmetric state, referred to as the $B^{\pm}$ state, and the para state (I_2 = 0) is assigned to the spatially symmetric state, referred to as the A^+ state. A^+ is the ground state and $B^{\pm}$ is the vibrationally excited state of the internal rotation of the H_2O moiety, using the notation of the group G_4 (*19*) without considering the symmetry of the electronic state. Because the present study cannot differentiate between B^+ and B^-, the excited state is referred to as the $B^{\pm}$ state. Two *b*-type transitions for the A^+ and $B^{\pm}$ states have also been observed with the double-resonance technique by monitoring the *a*-type transitions with $N = 2 \leftarrow 1$ (fig. S1). Observed rotational transitions and the energy level diagram are shown in Fig. 3, and all the observed transition frequencies are listed (tables S1 and S2). The spectral carrier was confirmed to be H_2O–HO_2 based on the following evidence: All of the observed transitions show paramagnetic behavior and disappear in the absence of electric discharge or when a sample gas lacking either H_2O or O_2 was used, but they do not change their intensity substantially when the buffer gas is changed. The determined rotational constants agree reasonably well with those of the ab initio calculations (*17*), and the fine and hyperfine splitting patterns are consistent with those expected for H_2O–HO_2.

All of the observed transitions for the A^+ and $B^{\pm}$ states were subjected to least squares analyses using the Watson's *A*-reduced Hamiltonian including the fine and hyperfine interaction terms (*23*). The fits gave reasonable standard deviations: 11 kHz for the A^+ state and 12 kHz for the $B^{\pm}$ state, respectively. The determined molecular constants are listed in table S3, and the rotational constants are listed in Table 1. The theoretical rotational constants at the equilibrium structure differ from the experimental values by about 5% (Table 1); this deviation is mainly due to the large-amplitude internal motions with small barriers. To confirm this point, rotational constants at the transition states for the internal motions, E^*

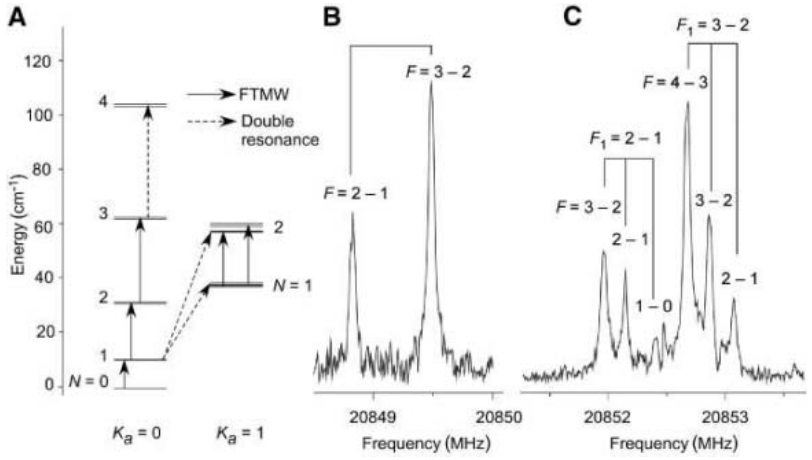

Fig. 3. (**A**) The rotational energy levels and the observed transitions of the H_2O–HO_2 complex in the A^+ state. The arrows with solid and dashed lines indicate the transitions observed by the FTMW spectrometer and the double-resonance technique, respectively. The energy diagram and the observed transitions of the B^+ state are similar to those of the A^+ state. FTMW spectra of $N_{K_a,K_c} = 2_{02} - 1_{01}$, $J = 2.5 - 1.5$ in the (**B**) A^+ state and (**C**) the B^+ state, which were obtained by averaging 140 and 370 free induction decay signals, respectively, are also shown. The hydrogen nucleus of the HO_2 moiety gives larger hyperfine splittings (F_1), and those of the H_2O moiety give smaller splittings (F) in the B^+ state.

Table 2. Comparison of the principal axis values of the magnetic dipole interaction tensor (in MHz).

	H_2O-HO_2	HO_2*
T_{xx}†	31.3	26.7
T_{yy}†	−20.4	−15.1
T_{zz}†	−10.9	−11.6

*Derived from the values in (*27*). †Because the off-diagonal components T_{ab} were determined experimentally, the principal axis values of the **T** tensor have been obtained by diagonalization. The *x* axis forms a 6° and 8° angle with the HO bond of HO_2 for the isolated HO_2 radical and the H_2O-HO_2 complex, respectively; the *z* axis is perpendicular to the molecular plane for the isolated HO_2 radical and almost perpendicular to the H_1-O_1-H_3-O_2-O_3 ring plane (shown in Fig. 1) for the H_2O-HO_2 complex.

and (12)* (Fig. 2, TS1 and TS2, respectively), were calculated (Table 1). The experimentally determined rotational constants have intermediate values between these calculated values.

The theoretical calculations show that the HO_2 monomer and the OH portion of H_2O form an almost-planar five-membered ring with one H atom sticking out from the ring (Fig. 1). The nonplanarity of the complex is quantified by the inertial defect, $\Delta I = I_{cc} - I_{bb} - I_{aa}$, which is calculated to be –1.0357 atomic mass unit (amu) Å^2 from the theoretical rotational constants at the equilibrium structure. The experimental rotational constants yield a ΔI value of –0.0922 amu Å^2, much smaller in its absolute value than was predicted from the ab initio structure. Thus, the vibrationally averaged structure is more nearly planar than the theoretical equilibrium structure.

The hydrogen bond length, $R(H_3 \cdots O_1)$, is calculated to be 1.875 Å from $(B + C)/2$, where B and C are the experimental rotation constants, by fixing other geometrical parameters to the theoretical results. Although this value is longer than the ab initio value (1.795 Å), presumably due to the large-amplitude motions, it is much shorter than those of typical hydrogen-bonded complexes; e.g., 2.019 Å for the water dimer (*24*) and 1.945 Å for the H_2O–HO radical complex (*12*). This feature may be because of the relative strength of the hydrogen bond of the H_2O–HO_2 complex, for which the ab initio binding energy (D_e) of 9.4 kcal/mol (*3*) is much larger than those of the water dimer [5.02 kcal/mol (*25*)] and the H_2O–HO complex [5.6 kcal/mol (*26*)]. The accuracy of this calculated binding energy is supported by close agreement between measured centrifugal distortion constants [Δ_N = 37.45(11) kHz and δ_N = 6.43(14) kHz, where the values in parentheses indicate one standard deviation of the least squares analysis and apply to the last digits of the constants] and the values calculated using the ab initio potential energy surface (Δ_N = 39.51 kHz and δ_N = 6.69 kHz). Although X–H···Y bonds are almost linear for most characterized hydrogen-bonded complexes, the O_2–$H_3 \cdots O_1$ bond of H_2O–HO_2 is significantly bent [$\angle(O_2H_3O_1)$ = 148.6°], where atoms are numbered as shown in Fig. 1. An attractive force between $O_3 \cdots H_1$ may contribute to this bent structure.

The Fermi coupling constant, a_F, is an important parameter for probing the effect of complex formation on the unpaired electron distribution, because it gives a direct measure of the unpaired electron density at each hydrogen nucleus. The value for the HO_2 proton, $a_F(H_3)$ = –27.511(21) MHz, is almost identical to that of free HO_2, –27.518(75) MHz (*27*). Conversely, a_F for the hydrogen nuclei of the H_2O moiety, –0.0395(62) MHz, is very small. Thus only a small portion of the unpaired electron density is delocalized on the H_2O moiety. This distribution resembles the electronic structure of Ar–HO_2 (*28*), but differs significantly from those of other radical-molecule complexes such as H_2O–HO (*12*, *13*) and NO–HF (*10*), for which substantial delocalization of the unpaired electrons was observed. Despite minimal change in the unpaired electron distribution when the complex is formed, the binding energy of H_2O–HO_2 is much larger than that of H_2O–HO.

The principal axis values of the magnetic dipole coupling tensor of the HO_2 proton, $\mathbf{T}(H_3)$ (Table 2), can be related to those of isolated HO_2 by considering an axis rotation upon complex formation. This observation also indicates that the change in unpaired electron distribution upon complex formation is small. Furthermore, this analysis gives more detail on the geometry of the complex. The angle of rotation of HO_2 around the *c* axis (out of plane axis) upon complex formation is estimated to be 100°, by assuming that complexation does not change the direction of the principal axis of the dipolar coupling tensor to the HO_2 moiety, with the HO_2 moiety lying in the *ab* plane of the inertial axis system. This angle agrees well with the geometry predicted by the ab initio calculations. A similar analysis can be applied to the spin-rotation coupling constants (*23*).

The *a* axis component of the dipole moment, μ_a = 2.65 D, is calculated to be much larger than the *b* axis component, μ_b = 0.25 D, at the equilibrium structure of H_2O–HO_2 at the RCCSD(T)/aug-cc-pVTZ level. These values are consistent with the behavior observed in the present study; *a*-type transitions were saturated much more easily by the double-resonance experiment. The molecular constants determined here are sufficiently precise to predict transitions in the millimeter and submillimeter wave regions for direct atmospheric detection. Such observations would clarify roles of this complex in atmospheric HO_x chemistry. Moreover, reaction dynamics studies of HO_2 are expected to clarify the significance of H_2O–HO_2 in combustion chemistry. Given the signal-to-noise ratios of the observed spectra, larger complexes $(H_2O)_n$–HO_2 ($n \geq 2$) could be observed by using a similar experimental method, and their roles in atmospheric chemistry (*7*) could be subsequently investigated.

References and Notes

1. E. J. Hamilton Jr., *J. Chem. Phys.* **63**, 3682 (1975).
2. E. J. Hamilton Jr., R.-R. Lii, *Int. J. Chem. Kinet* **9**, 875 (1977).
3. S. Aloisio, J. S. Francisco, *J. Phys. Chem. A* **102**, 1899 (1998).
4. N. Kanno, K. Tonokura, A. Tezaki, M. Koshi, *J. Phys. Chem. A* **109**, 3153 (2005).
5. S. Aloisio, J. S. Francisco, R. R. Friedl, *J. Phys. Chem. A* **104**, 6597 (2000).
6. B. Nelander, *J. Phys. Chem.* **101**, 9092 (1997).
7. S. D. Belair, H. Hernandez, J. S. Francisco, *J. Am. Chem. Soc.* **126**, 3024 (2004).
8. R. S. Zhu, M. C. Lin, *Chem. Phys. Lett.* **354**, 217 (2002).
9. J. M. Hutson, *Annu. Rev. Phys. Chem.* **41**, 123 (1990).
10. C. R. Dennis, C. J. Whitham, B. J. Howard, *J. Chem. Phys.* **115**, 1367 (2001).
11. Y. Kasai, Y. Sumiyoshi, Y. Endo, *55th International Symposium on Molecular Spectroscopy* (Ohio State University, Columbus, OH, 2000).

12. Y. Ohshima, K. Sato, Y. Sumiyoshi, Y. Endo, *J. Am. Chem. Soc.* **127**, 1108 (2005).
13. C. S. Brauer *et al.*, *Chem. Phys. Lett.* **401**, 420 (2005).
14. M. I. Lester, B. V. Pond, D. T. Anderson, L. B. Harding, A. F. Wagner, *J. Chem. Phys.* **113**, 9889 (2000).
15. Y. Sumiyoshi, H. Katsnuma, K. Suma, Y. Endo, *J. Chem. Phys.* **123**, 054324 (2005).
16. K. Suma, Y. Sumiyoshi, Y. Endo, *J. Chem. Phys.* **121**, 8351 (2004).
17. The Gaussian 03 suite of programs (*29*) and Molpro 2002.6 suite of programs (*30*) were used for ab initio calculations. The centrifugal distortion constants were obtained by B3LYP/aug-cc-pVTZ [the Becke 3-parameter-Lee-Yang-Parr density functional method using Dunning's correlation-consistent polarized triple split valence basis set with diffuse functions (*18*)] of Gaussian 03. Other ab initio calculations with SCF, B3LYP, and RCCSD(T) (the restricted single and double excitation coupled-cluster approach with noniterative perturbational treatment of triple excitations) were performed using Molpro 2002.6.
18. R. A. Kendall, T. H. Dunning Jr., R. J. Harrison, *J. Chem. Phys.* **96**, 6796 (1992).
19. H. C. Longuet-Higgins, *Mol. Phys.* **6**, 445 (1963).
20. The character table of the group G_4 is given in (*23*).
21. Y. Endo, H. Kohguchi, Y. Ohshima, *Faraday Discuss.* **97**, 341 (1994).
22. M. Iida, Y. Ohshima, Y. Endo, *J. Chem. Phys.* **94**, 6989 (1991).
23. Materials and methods are available as supporting material on *Science* Online.
24. J. A. Odutola, T. R. Dyke, *J. Chem. Phys.* **72**, 5062 (1980).
25. W. Klopper, J. G. C. M. van Duijneveldt-van de Rijdt, F. B. van Duijneveldt, *Phys. Chem. Chem. Phys.* **2**, 2227 (2000).
26. A. A. Nanayakkara, G. G. Balintkurti, I. H. Williams, *J. Phys. Chem.* **96**, 3662 (1992).
27. K. V. Chance *et al.*, *J. Mol. Spectrosc.* **183**, 418 (1997).
28. K. Suma, W. Funato, Y. Sumiyoshi, Y. Endo, *J. Chem. Phys.* **122**, 184302 (2005).
29. M. J. Frisch *et al.*, Gaussian 03, Revision C.02, Gaussian, Inc., (Wallingford, CT, 2004).
30. Molpro is a package of ab initio programs written by H.-J. Werner *et al.*.
31. This work was supported by a Grant-in-Aid for priority research entitled "Radical Chain Reactions" (grant no.13127101). K.S. acknowledges the support of the Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (JSPS No. 16-10895).

Supporting Online Material

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Materials and Methods
Tables S1 to S4
Fig. S1
References

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Early Maya Writing at San Bartolo, Guatemala

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The ruins of San Bartolo, Guatemala, contain a sample of Maya hieroglyphic writing dating to the Late Preclassic period (400 B.C. to 200 A.D.). The writing appears on preserved painted walls and plaster fragments buried within the pyramidal structure known as "Las Pinturas," which was constructed in discrete phases over several centuries. Samples of carbonized wood that are closely associated with the writing have calibrated radiocarbon dates of 200 to 300 B.C. This early Maya writing implies that a developed Maya writing system was in use centuries earlier than previously thought, approximating a time when we see the earliest scripts elsewhere in Mesoamerica.

Research on the origins of Maya hieroglyphic writing has long been hindered by the paucity of good archaeological contexts and reliable dates for inscribed artifacts and monuments. With a few exceptions, examples of archaic Maya script appear on illicitly excavated objects that can be stylistically dated to no earlier than about 100 B.C. to 100 A.D., when writing seems to have been already well established elsewhere in Mesoamerica. Here we provide new evidence of early Maya writing preserved in the ruins of San Bartolo, Guatemala.

The ruins of San Bartolo, Guatemala (Fig. 1) were identified in 2001 and include early wall paintings buried within a pyramidal structure today known as "Las Pinturas." These had been partially exposed by illicit digging a few years previously, and subsequent scientific excavations in Room 1 (as that location is now designated) have uncovered most of this important mural, dating to ~100 B.C. (*1*–*4*) (figs. S6 to S10). Tunneling deeper into the Las Pinturas structure has since led to the discovery of other buildings with remains of painted decoration that are substantially older than the Room 1 murals.

One example of this earlier painting comes from a block from a dismantled wall of the building that once stood on the platform of the Sub-V construction phase (Fig. 2). The Room 1 murals were painted on the Sub-I phase of the pyramid, that is, four construction episodes later than the Sub-V phase. The ~4-m-high Sub-V platform extends 28 m by 12 m at its base and supported three separate masonry rooms. The 2005 excavations established that its central room was richly decorated and painted with polychrome murals. The surviving doorjamb bears a colorful image of the Maize God, who is a central character in the mythological scenes of the later Room 1 murals (*4*). The line of script was possibly associated with this religiously themed scenery, but its original placement within the room is uncertain.

We obtained accelerator mass spectrometry (AMS) radiocarbon dates on five charcoal samples from sealed deposits in the three architectural strata (Sub-VI, Sub-V, and Sub-IV) in order to bracket the age of the painted blocks (Fig. 3). The first of these—from within the floor of the Sub-VI platform, the construction phase that was encapsulated by Sub-V construction—provides a maximum uncalibrated radiocarbon date of 2260 ± 40 years before present (years B.P.) [400 to 200 B.C.; 2σ (95% probability)

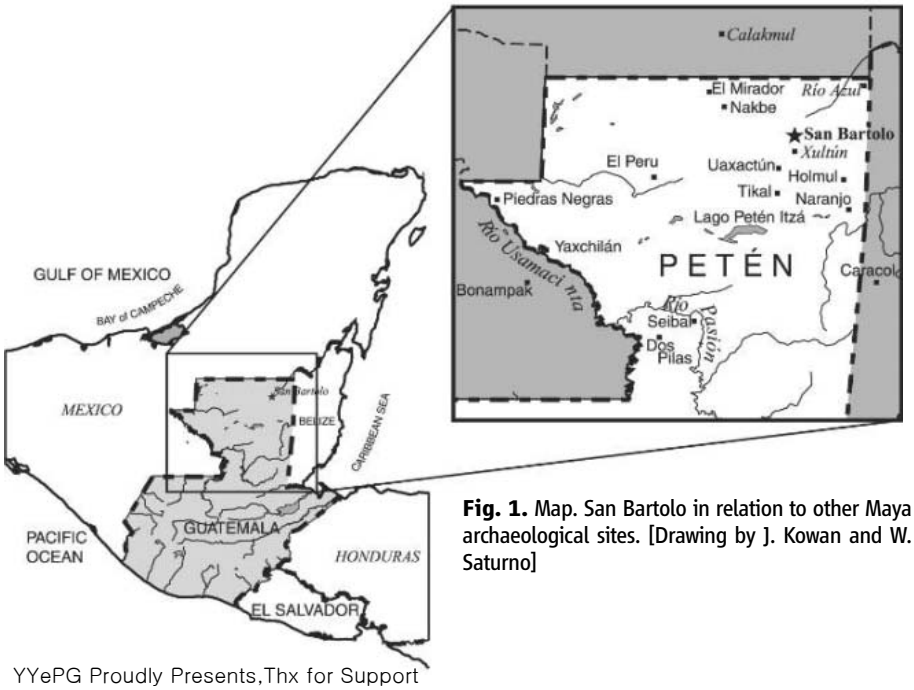

Fig. 1. Map. San Bartolo in relation to other Maya archaeological sites. [Drawing by J. Kowan and W. Saturno]

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Fig. 2. Glyph block. The Sub-V painted block in situ. [Photograph by B. Beltrán]

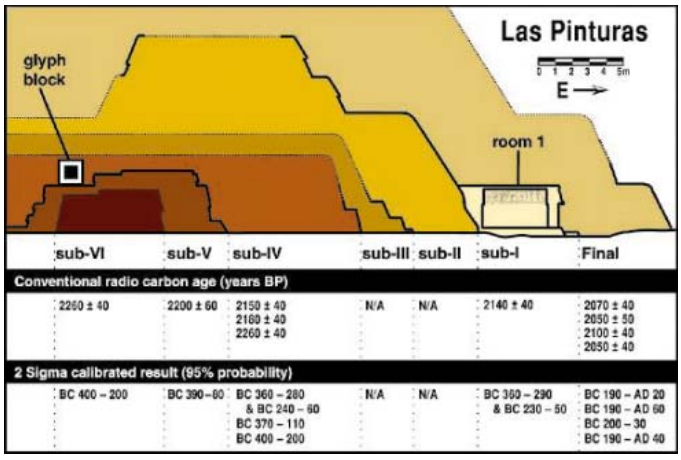

Fig. 3. Las Pinturas. Architectural profile illustrating AMS radiocarbon dates for the construction sequence of the structure, the location of the Sub-V building phase, the painted glyph block, and the Room 1 mural. Scale in meters. [Drawing by J. Kowan and W. Saturno]

calibrated range] (fig. S1). A sample from within the floor of Sub-V dates the construction of the room at 2200 ± 60 years B.P. uncalibrated [390 to 80 B.C.; 2σ (95% probability) calibrated range] (fig. S2). The final three samples with dates of 2260 ± 40 years B.P. uncalibrated [400 to 200 B.C.; 2σ (95% probability) calibrated range], 2180 ± 40 years B.P. uncalibrated [370 to 100 B.C.; 2σ (95% probability) calibrated range], and 2150 ± 40 years B.P. uncalibrated [360 to 60 B.C.; 2σ (95% probability) calibrated range] (figs. S3 to S5) surround the painted blocks and relate contextually to both the destruction of the Sub-V painted room and the subsequent construction of the Sub-IV platform above it. Taken together, these samples and those analyzed in association with the final two phases of construction imply that the text was painted between 300 and 200 B.C.

The painted block bears a column of 10 hieroglyphs (Fig. 4). The text appears to be the end of a longer sequence of signs that continued above. All are painted in a thick black line on white plaster, apparently along a subtle pinkish-orange stripe that served as a guideline for the scribe. As with later examples of Maya writing discovered at San Bartolo, its decipherment remains a challenge (*4*). Later texts from the Room 1 murals are only partially readable, because sign forms appear considerably different from the familiar elements of later Maya script. The San Bartolo Room 1 paintings date centuries before the first fully legible Maya writing from around 250 to 300 A.D., and the signs of the Sub-V block are older still, containing archaic forms.

The one fully recognizable glyph (pA7) is an early version of the sign that reads AJAW, a ubiquitous title in Maya texts that means "lord," "noble," or "ruler." It evidently formed part of a more extended title phase in reference to some person, either historical or mythical. Some signs have qualities that might be vaguely pictorial, such as pA2 with its suggestion of a hand holding a brush or alternatively a sharp bloodletter. Other signs are more abstract-looking forms, probably ancestral to components of later Maya script. In their overall appearance, the text bears some resemblance to the so-called Epi-Olmec script used by neighboring peoples to the west during the Late Preclassic and Early Classic periods (*5*, *6*). All examples of that script postdate the San Bartolo block, however, raising the question of the direction in which any influence may have flowed.

Preclassic writing from the Maya area is scarce and has been difficult to date accurately. Most other examples are known from stone monuments found in surface or near-surface contexts or from illicitly excavated portable objects. One notable early inscription from El Mirador probably dates to no earlier than 100 BC on the basis of stylistic comparisons (*7*). Another carved monument with glyphs from El Portón, Guatemala, may date to the first two or thee centuries B.C., on the basis of a single radiocarbon date not in direct association with the stone (*8*). The newly discovered San Bartolo text can now be firmly dated to the same general period, and its fine preservation offers an unusual look at the form that Maya script assumed in its early history.

Fig. 4. Painted hieroglyphs. Scale drawing of Sub-V painted glyph block. Glyphs assigned preliminary column and row designations. Scale in centimeters (pA 1 to 10). [Drawing by D. Stuart]

The San Bartolo text raises the question of the relation between Maya writing and other early script traditions in Mesoamerica. In the Preclassic era, writing systems were firmly established by about 400 B.C. among complex cultures in what is now Oaxaca and perhaps in the Isthmus of Tehuantepec (*9*–*12*), although the dating of evidence for this remains controversial (*13*–*15*). It now appears that the Maya also participated in the Preclassic cultures of literacy, and at a much earlier date than previously believed.

References and Notes

1. W. Saturno, *Symbols* (Peabody Museum of Archaeology and Ethnology, Harvard University, Fall 2002), p. 3.
2. W. Saturno, D. Stuart, H. Escobedo, I. Graham, "Reonocimiento Arqueológico y Conservación de San Bartolo, Guatemala" (Instituto de Antropología e Historia, Guatemala City, Guatemala, 2001).
3. T. O'Neill, *Natl. Geogr. Mag.* **201**, 70 (2002).
4. W. Saturno, K. Taube, D. Stuart, *The Murals of San Bartolo, Guatemala*, Part 1, *The North Wall* (Publ. 7, Ancient America, Barnardsville, NC, 2005).
5. F. Winfield Capitaine, *La Estela 1 de La Mojarra, Veracruz* (Publ. 16, Research Reports on Ancient Maya Writing, Washington, DC, 1988).
6. J. S. Justeson, T. Kaufman, *Science* **259**, 1703 (1993).
7. R. D. Hansen, *An Early Maya Text from El Mirador, Guatemala* (Research Reports on Ancient Maya Writing, Washington, DC, 1991).
8. R. J. Sharer, D. W. Sedat, *Archaeological Investigations in the Northern Maya Highlands* (University Museum, Univ. of Pennsylvania, 1987), pp. 49–73.
9. A. Caso, *Handb. Mid. Am. Indians* **3**, 931 (1965).
10. J. Marcus, *Annu. Rev. of Anthropol.* **5**, 35 (1976).
11. J. Marcus, *Mesoamerican Writing Systems: Propaganda, Myth and History in Four Ancient Civilizations* (Princeton Univ. Press, Princeton, NJ, 1992), pp. 41–42.
12. M. E. D. Pohl, K. O. Pope, C. von Nagy, *Science* **298**, 1984 (2002).
13. R. Cahn, M. Winter, *Indiana* **13**, 39 (1993).
14. S. D. Houston, in *First Writing: Script Invention as History and Process* (Cambridge Univ. Press, Cambridge, 2005), p. 293.
15. K. V. Flannery, J. Marcus, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 11801 (2003).
16. Excavations by the Proyecto San Bartolo between 2002 and 2005 were supported by grants from the National Endowment for the Humanities (grant RZ- 50086); National Geographic Society, Committee for Research and Exploration (grants 7065-01, 7222-02, 7393-03, 7601-04, and

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Figs. S1 to S10
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Toward Automatic Reconstruction of a Highly Resolved Tree of Life

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We have developed an automatable procedure for reconstructing the tree of life with branch lengths comparable across all three domains. The tree has its basis in a concatenation of 31 orthologs occurring in 191 species with sequenced genomes. It revealed interdomain discrepancies in taxonomic classification. Systematic detection and subsequent exclusion of products of horizontal gene transfer increased phylogenetic resolution, allowing us to confirm accepted relationships and resolve disputed and preliminary classifications. For example, we place the phylum Acidobacteria as a sister group of δ-Proteobacteria, support a Gram-positive origin of Bacteria, and suggest a thermophilic last universal common ancestor.

Reconstructing the phylogenetic relationships among all living organisms is one of the fundamental challenges in biology. Numerous attempts to derive a tree of life using various methods have been published [for a review, see (*1*)], and its principal existence has been questioned recently (*2*, *3*). Moreover, even under the assumption of a tree of life, numerous groupings and taxonomic entities still remain heavily debated, and the advent of molecular and genomic data has increased the variety of classifications rather than reducing the problem (*1*). Theoretical and practical limits to reconstructing a tree of life have been put forward, such as the insufficient amount of discriminating characters available, even in information-rich genomic data sets (*4*), and the computing resources required to cope with large numbers of species (*1*). Furthermore, there are factors that hamper accurate reconstruction of phylogenetic trees regardless of the methods used, such as sampling biases of species included (*5*) and dilution of phylogenetic signal by horizontal gene transfer (HGT) (*6*), the

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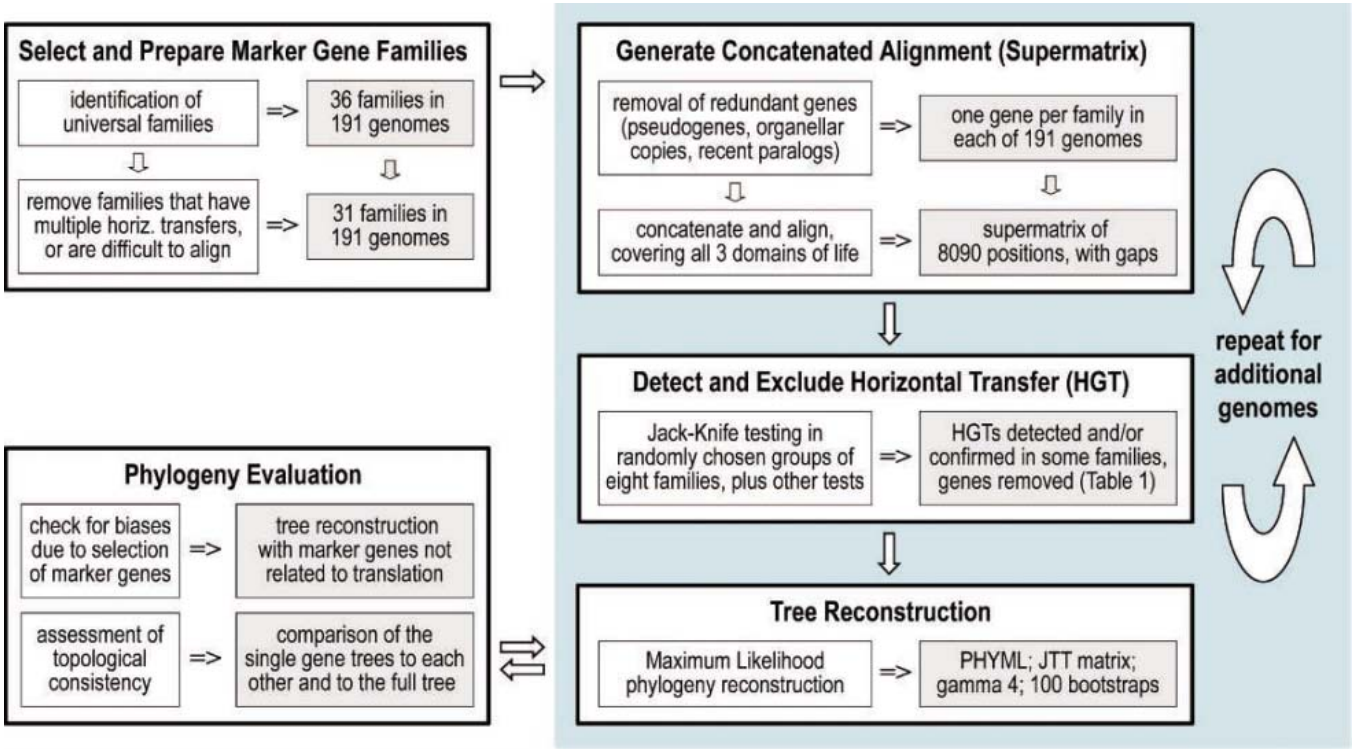

Fig. 1. Overview of the procedure. The white boxes represent the major steps for building the pan-domain phylogeny presented here. Steps in gray represent automatable parts of the procedure that need to be carried out for including further species. For the 31 clusters of orthologous groups (COGs) used in the analysis, we manually derived 1:1 orthologs by removing mitochondrial and chloroplast paralogs from corresponding multiple alignments. We built domain-specific alignments by using corresponding proteins encoded by the 31 orthologs and aligned the resulting profiles. With this procedure, we maximized the number of positions of the global alignment and reduced the number of misaligned residues. For a detailed description of the methods, see (*8*).

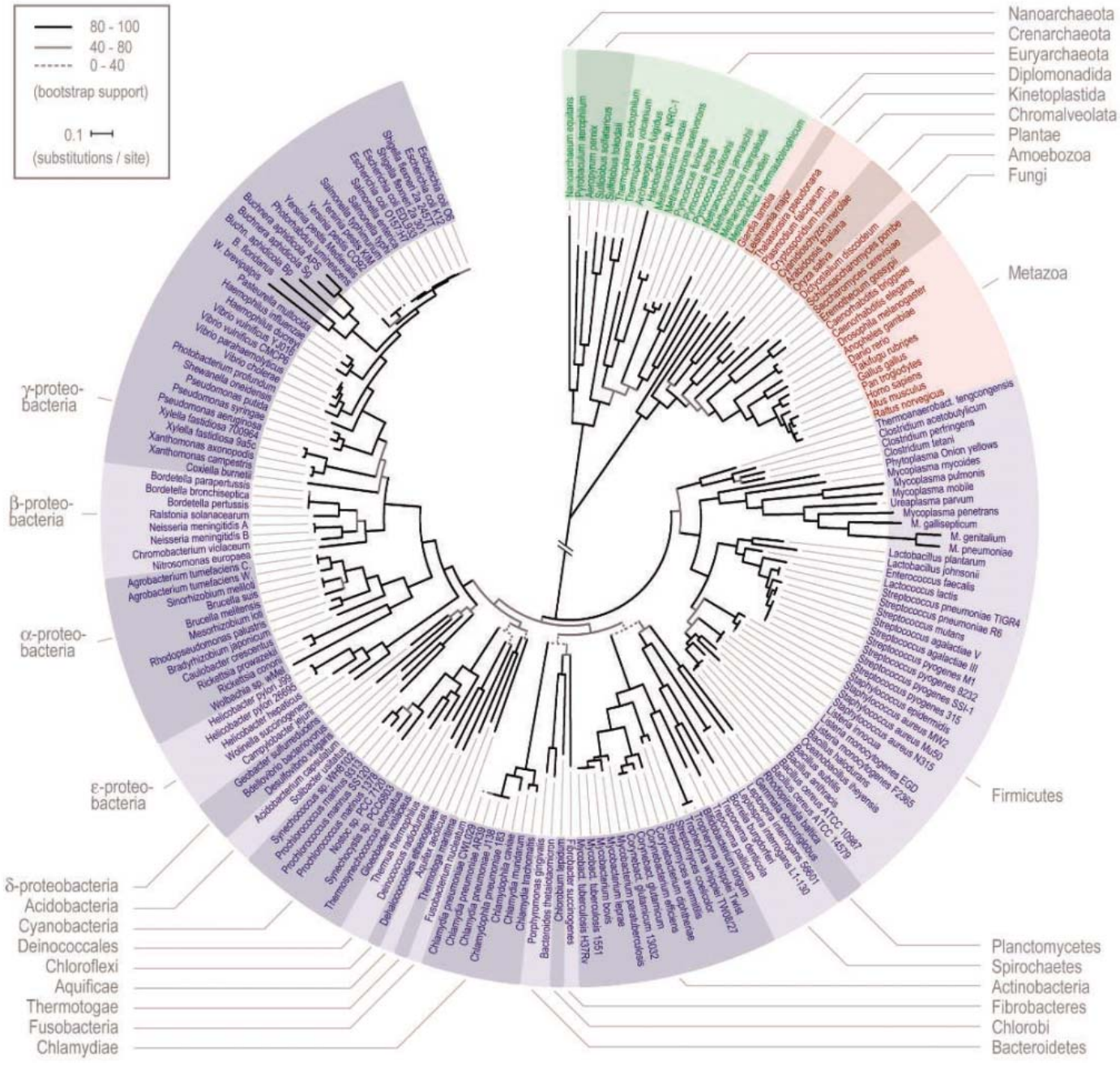

Fig. 2. Global phylogeny of fully sequenced organisms. The phylogenetic tree has its basis in a cleaned and concatenated alignment of 31 universal protein families and covers 191 species whose genomes have been fully sequenced (*14*). Green section, Archaea; red, Eukaryota; blue, Bacteria. Labels and color shadings indicate various frequently used subdivisions. The branch separating Eukaryota and Archaea from Bacteria in this unrooted tree has been shortened for display purposes.

extent of which is still extremely controversial (*2*, *3*, *7*). In addition to these difficulties, different data sets have been used with a variety of methods and parameter settings, making it almost impossible to quantitatively compare the proposed results. Hence, there exists the challenge and requirement for a reproducible and updatable pipeline to reconstruct the tree of life by means of a commonly available data set, such as completely sequenced genomes. Here, we demonstrate the feasibility of the tree construction and present a phylogeny based on an alignment of sufficient length and resolution to accurately calculate comparable branch lengths across all three domains of life. We have created for this purpose a supermatrix of 31 concatenated, universally occurring genes with indisputable orthology in 191 species with completely annotated genomes (Fig. 1 and table S1). Although initial identification and analysis of these genes required considerable manual effort (*8*), the inclusion of additional species with completely annotated genomes has pipeline character (Fig. 1). Because the 31 universal genes are all involved in translation, we applied the same tree-building procedure to independent sets of domain-specific nontranslational genes (*8*).

For the tree reconstruction, we mostly used standard approaches (Fig. 1) with the exception of a procedure for detection and selective exclusion of HGTs, which turned out to be essential for obtaining a highly resolved tree. We started with 36 genes universally present in all 191 species for which orthologs could be unambiguously identified (*8*) and eliminated five of them from the analysis (mostly tRNA synthetases) because they

have undergone multiple horizontal transfers and/or were difficult to align (Fig. 1 and table S1). Although the 31 remaining genes are unlikely to be subjected to lateral transfers because they mainly encode for ribosomal proteins (*9*), we systematically tested them for any HGT event not yet identified. We randomly allocated the 31 gene products into four groups, and for each group we derived the corresponding subsets of trees where each protein was in turn missing from the alignment (resampling with displacement: jackknife test). We subsequently checked for topological incongruence within each subset of trees and further tested candidate HGTs by two other independent measures (*8*). If at least one of these two measures could confirm the jackknife indication, the gene was considered horizontally transferred and removed from the corresponding alignment (Fig. 1 and table S2).

Our approach [confirmed by single tree analysis (*8*)] detected a total of 7 HGT candidates [i.e., orthologous gene displacements (*10*)] among 31 orthologs from 191 species, with some species being involved in more than one HGT event (table S2). Three out of the four aminoacyl-tRNA synthetases (aa-RSs) used in this analysis have undergone HGT, including Valyl-RS (COG0525), which had been reported before (*11*), thus confirming the mobility of these enzymes (*12*). Clostridia is the only class that acquired ribosomal proteins by lateral transfer, likely in a single ancient event, because the displaced orthologs are present in all sequenced Clostridia species (table S2). To our knowledge, only one other horizontal transfer of ribosomal proteins has been reported so far (*13*). The identification of 7 HGTs in the 31 translation-related genes compares with the 30 (10 per domain) lateral transfers detected in domain-specific trees from 24 nontranslational genes (*8*).

Species-specific exclusion of HGTs and concatenation of all gene product alignments resulted in a supermatrix of 8090 positions for 191 species. This supermatrix was subsequently used to reconstruct the tree of life shown in Fig. 2 (*14*).

The global tree topology was supported by two independent measurements: First, by using domain-specific subtrees from nontranslational genes we could confirm the monophyly of all major divisions and reproduce most of their branching orders (*8*), albeit with weaker statistical support. This is due to lower sequence coverage and/or conservation as well as a higher number of excluded characters because of the higher incidence of HGTs (*8*). Secondly, three independent tests carried out on individual gene trees revealed that, although they are not identical, they share similarities with both the obtained tree of life and with each other (*8*). Although it may be possible to reject the null hypothesis of each of these tests without much difficulty, their combined evidence suggests that the gene trees have a cohesive phylogenetic signal.

Within the tree of life, as many as 65% of the branches are supported by a bootstrap proportion (BP) of 100%, and 81.7% have more than 80% BP support, enabling us to propose resolutions to debated classifications at both the root and the tips of the tree (Table 1). Although in Prokaryota statistical support for deeper branches is generally weaker than that for the recent ones, it is noteworthy that, within Bacteria, the Firmicutes appear to comprise the earliest branching phylum, in agreement with a proposed Gram-positive ancestor for all Bacteria (*15*) (Fig. 2 and Table 1). In our tree, Firmicutes are placed at the earliest division of Eubacteria with 66% BP support, and 33% of remaining BP show at least a subclade of Firmicutes at the earliest division. This placement and the fact that the 15 slowest evolving taxa of the Bacteria are all Gram positive (*8*) support the theory of a Gram-positive origin of Bacteria. Furthermore, the thermophilic Firmicute *Thermoanaerobacter tengcongensis* is the taxon with the shortest overall phylogenetic distance to the root of Bacteria (Fig. 2) and as such is most likely to have retained ancestral states (*16*). Together with the fact that slowest evolving, ancestral Archaea (table S7) are also (hyper)thermophilic (*8*), this lends support to the hypothesis that the last universal common ancestor was living at high temperatures.

At the base of the Proteobacteria, the monophyletic Acidobacteria appear as a sister group to the δ-Proteobacteria (Fig. 2). The 64% BP support for this relationship indicates that the Acidobacteria may be a sixth divergent class within Proteobacteria. The Proteobacterial-Acidobacterial monophyly is supported with a BP of 98%, further raising the question whether Acidobacteria should indeed be an independent phylum (*17*).

Toward the tips of the tree, within Cyanobacteria *Synechococcus* (sp.WH8102) groups with *Prochloroccales* and *Nostoc* groups with *Synechocystis*, a result that has been supported by some ribosomal RNA (rRNA) studies (*18*) and challenges the classical order Chroococcales (*19*).

Within Archaea, the position of Nanoarchaeota remains debated [e.g., (*20*)]. We find (with 100% BP support) that they are a sister group of Crenarchaeota, without an indication of reported HGTs from Crenarchaeota (*20*) in all core genes studied.

Within Eukaryota, our tree gives clear support for the classical Coelomata hypothesis that groups Arthropoda with Deuterostomia (chordates) in a monophyletic clade. This is in contrast to the "new animal phylogeny" that groups nematodes and arthropods into the monophyletic Ecdysozoa (*21*, *22*). The ecdysozoan clade has been supported by small subunit rRNA and single-gene phylogenies [(*23*) and references therein] but has been rejected by a number of recent studies on the basis of genomics features and whole-genome phylogenies [(*24*) and references therein]. Current sampling biases and accelerated evolution of se-

Table 1. Noteworthy selected features of the tree of life phylogeny that are novel, debated, or difficult to reproduce according to current literature. An extended version of the table is available as table S6. In the case of Firmicutes as the earliest branching bacterial phylum, it is noteworthy that the remaining 33% of the BP show at least a subclade of the Firmicutes at the earliest division.

Domain	Topological feature	BP (%)
	Coelomata hypothesis	100
Eukaryota	Amoebozoa related to Opistokonta	41
	Deep branching of Diplomonadida	100
	Relationships within phyla	
	Separation between β- and γ-Proteobacteria	100
	Disruption of Chroococcales monophyly	100
	Disruption of Actinomycetales monophyly	100
	Acidobacteria-Proteobacteria clade	98
	Cluster of *F. succinogenes* next to the Chlorobium-Bacterioidales (Sphingobacteria hypothesis)	62
	Cluster of *F. nucleatum* with hyperthermophylic Bacteria	36
Eubacteria	*Relationships between phyla*	
	Grouping of Chlamydiae, Spirochetes, Actinobacteria, and Bacteriodales-Chlorobi	67
	Grouping of Cyanobacteria, hyperthermophylic, and Deinococcales-Chloroflexi	51
	Relationships between super-phyla	
	Grouping of Proteobacteria with Cyanobacteria, hyperthermophylic, and Deinococcales-Chloroflexi	74
	Deep branching of Firmicutes	66
	Relationship within phyla	
	A. fulgidus with halobacterium and methanosarcina	99
Archaeabacteria	*Relationship between phyla*	
	Nanoarchaea as a sister branch of Crenarchaea	100

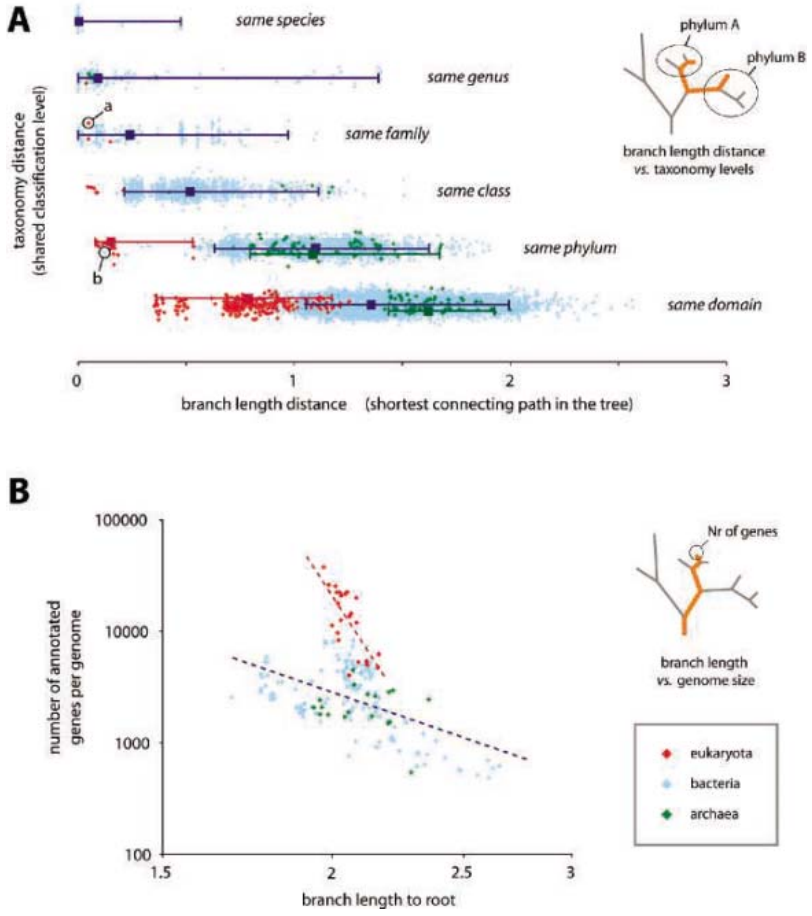

Fig. 3. Global analysis of branch length information. **(A)** Average sequence divergence within taxonomic classification units. Each data point denotes a pairwise comparison of two taxa, relating their intertaxa branch-length distance (i.e., sequence divergence) with their level of relatedness according to the National Center for Biotechnology Information taxonomy ("taxonomy distance"). Horizontal bars denote 95% intervals and medians of the data. Some minor taxonomy hierarchy levels have been omitted. Marked items: (Point a) *Homo sapiens* versus *Pan troglodytes*. The sequence divergence between human and chimp is low; they most likely would have been assigned the same genus if they had been prokaryotes [see also (*30*) for a proposed revision]. (Point b) *Synechococcus* (sp. WH8102) versus *Prochlorococcus marinus 9313*. The two species are annotated as distinct orders, but they appear quite closely related, challenging the classical order of Chroococcales. **(B)** Evolutionary speed and genome size. For each taxon, cumulative branch lengths from the tip to the root is plotted against genome size (measured here as number of genes). Dashed lines are linear regressions.

quenced representatives of certain metazoan lineages (e.g., arthropods and nematodes) (Fig. 2) may factor in these results. This highlights the need for the sequencing of slow-evolving species (*16*), which may resolve such controversies in the tree.

Despite a highly resolved and robust tree, we cannot exclude a few uncertainties in tree topology due to biased species sampling or long branch attraction (LBA) (*25*). For example, the grouping of *Thermotoga* and *Aquifex* in our and other trees might be partially caused by their common thermophilic life-styles (*26*), whereas LBA might account for the placement of diplomonadida (*Giardia lamblia*) as the most basal eukaryal taxon (Table 1).

The use of a common protein set across all three domains of life also ensures that the observed branch lengths are comparable across the entire tree. This enables, for example, an objective, quantitative analysis of the consistency of traditional taxonomic groupings (Fig. 3). As expected, the hierarchy of taxonomic groups correlates with phylogenetic diversity measured between and within them (e.g., species belonging to the same family have a shorter branch length distance than species belonging only to the same phylum). Within each taxonomic level, branch lengths distances vary considerably (*27*), apparently owing to factors that influence substitution rates, such as differences in life-style or population size. However, even when taking this effect into account, we observe a strong discrepancy between taxonomic divisions within Eukaryota and Prokaryota (Fig. 3A). Organisms that have been assigned to separate phyla in Eukaryota would clearly belong to the same phylum in the prokaryotic classification. Historically, eukaryotes have obviously been given more taxonomic resolution than prokaryotes, a testament to their greater morphological diversity.

Another universal trend is that smaller genomes evolve faster [i.e., have longer branch lengths (Fig. 3B)]. This has been noted before for pathogenic or endosymbiontic organisms with reduced genomes, and it is easily explained because they have only limited capabilities to remove mutations by means of recombination or DNA repair (*28*, *29*). However, we observe this trend also for genomes of larger sizes, including free-living prokaryotes and eukaryotes. Intriguingly, there is not a single organism sequenced that is fast-evolving and has a large genome (Fig. 3B). This suggests that the coupled processes of genome reduction and evolutionary acceleration may be irreversible: Genomes apparently do not grow again after a prolonged phase of genome reduction.

The pan-domain phylogeny that resulted from the procedure presented here will increase in resolution with more species being sequenced. This updatable reference phylogeny of completely sequenced species allows accurate comparisons of branch lengths across domains. The resulting tree of life will be an invaluable tool in many areas of biological research, ranging from classical taxonomy, via studies on the rate of evolution, to environmental genomics where DNA fragments of unknown phylogenetic origin need to be assigned.

References and Notes

1. F. Delsuc *et al.*, *Nat. Rev. Genet.* **6**, 361 (2005).
2. W. F. Doolittle, *Science* **284**, 2124 (1999).
3. J. P. Gogarten *et al.*, *Mol. Biol. Evol.* **19**, 2226 (2002).
4. J. R. Brown *et al.*, *Nat. Genet.* **28**, 281 (2001).
5. D. M. Hillis *et al.*, *Syst. Biol.* **52**, 124 (2003).
6. H. Philippe, C. J. Douady, *Curr. Opin. Microbiol.* **6**, 498 (2003).
7. V. Daubin, N. A. Moran, H. Ochman, *Science* **301**, 829 (2003).
8. Materials and methods are available on *Science* Online.
9. R. Jain *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3801 (1999).
10. E. V. Koonin *et al.*, *Trends Genet.* **12**, 334 (1996).
11. C. G. Kurland, S. G. E. Andersson, *Microbiol. Mol. Biol. Rev.* **64**, 786 (2000).
12. Y. I. Wolf *et al.*, *Genome Res.* **9**, 689 (1999).
13. C. Brochier *et al.*, *Trends Genet.* **16**, 529 (2000).
14. A detailed version of the tree in Fig. 2, including bootstrap support values and branch lengths, is available at www.bork.embl.de/tree_of_life/.
15. A. L. Koch, *Trends Microbiol.* **11**, 166 (2003).
16. F. Raible *et al.*, *Science* **310**, 1325 (2005).
17. P. Hugenholtz *et al.*, *J. Bacteriol.* **180**, 4765 (1998).
18. D. Honda *et al.*, *J. Mol. Evol.* **48**, 723 (1999).
19. K. W. Nägeli, *Allg. Schweiz. Ges. Gesammten Naturwiss.* **10**, 45 (1849).
20. C. Brochier *et al.*, *Genome Biol.* **6**, R42 (2005).
21. A. Adoutte *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4453 (2000).
22. K. M. Halanych, *Annu. Rev. Ecol. Evol. Syst.* **35**, 229 (2004).
23. H. Philippe *et al.*, *Mol. Biol. Evol.* **22**, 1246 (2005).
24. G. K. Philip *et al.*, *Mol. Biol. Evol.* **22**, 1175 (2005).
25. J. Felsenstein, *Syst. Zool.* **27**, 401 (1978).
26. D. P. Kreil, C. A. Ouzounis, *Nucleic Acids Res.* **29**, 1608 (2001).
27. K. T. Konstantinidis, J. M. Tiedje, *J. Bacteriol.* **187**, 6258 (2005).
28. N. A. Moran, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2873 (1996).
29. S. G. E. Andersson, C. G. Kurland, *Trends Microbiol.* **6**, 263 (1998).

30. D. E. Wildman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 7181 (2003).
31. We thank S. Hooper for help in designing trees and are grateful to members of the Bork group as well as T. Gibson, M. Gouy, M. Huynen, A. Budd, and M. Wolf for stimulating discussions. This work was supported in part by Bundesministerium fuer Bildung und Forschung (Nationales Genomforschungsnetz grant 01GR0454 to P.B.) and by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek.

Supporting Online Material
www.sciencemag.org/cgi/content/full/311/5765/1283/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 to S7
References and Notes

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Germline Mutations in Genes Within the MAPK Pathway Cause Cardio-facio-cutaneous Syndrome

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Cardio-facio-cutaneous (CFC) syndrome is a sporadic developmental disorder involving characteristic craniofacial features, cardiac defects, ectodermal abnormalities, and developmental delay. We demonstrate that heterogeneous de novo missense mutations in three genes within the mitogen-activated protein kinase (MAPK) pathway cause CFC syndrome. The majority of cases (18 out of 23) are caused by mutations in *BRAF*, a gene frequently mutated in cancer. Of the 11 mutations identified, two result in amino acid substitutions that occur in tumors, but most are unique and suggest previously unknown mechanisms of B-Raf activation. Furthermore, three of five individuals without *BRAF* mutations had missense mutations in either *MEK1* or *MEK2*, downstream effectors of B-Raf. Our findings highlight the involvement of the MAPK pathway in human development and will provide a molecular diagnosis of CFC syndrome.

There is an emerging group of medical genetic syndromes that are due to activating mutations in genes associated with the Ras pathway, including Noonan syndrome (NS, *PTPN11*) (*1*) and Costello syndrome (CS, *HRAS*) (*2*, *3*). Cardio-facio-cutaneous syndrome [CFC; Online Mendelian Inheritance in Man (OMIM) 115150] has many features that overlap with NS and CS. CFC is a sporadic, complex developmental disorder involving characteristic craniofacial features, cardiac anomalies (most commonly atrial septal defect and pulmonic stenosis), hair and skin abnormalities, postnatal growth deficiency, hypotonia, and developmental delay (*4*). Because of the similarity between CFC and CS, we screened patients with CFC for mutations in *HRAS* (*3*). We found no mutations in this gene, supporting a distinct genetic etiology between CS and CFC syndromes. We therefore expanded our search and sequenced other Ras genes (see Materials and Methods in the supporting online material), as well as genes encoding downstream effectors of Ras: *BRAF*, *CRAF*, *MEK1*, and *MEK2*. Our cohort consisted of 23 unrelated individuals with the clinical diagnosis of CFC syndrome who did not have a mutation in *HRAS* or *PTPN11* (table S1).

Using bidirectional sequencing of peripheral lymphocyte genomic DNA, we identified heterogeneous missense mutations in *BRAF* [GenBank accession (NM) 004333] in 18 out of 23 (78% of) individuals having CFC syndrome. Eleven distinct missense mutations clustered in two regions (Fig. 1A). Five individuals had a nucleotide (nt) switch, specifically nt770A→G transition in exon 6, with a predicted missense substitution of arginine for Gln^{257} (Q257R) (*5*) in the cysteine-rich domain (CRD) of the conserved region 1 (CR1) (Fig. 1B). The other cluster of mutations was in the protein kinase domain and involved exons 11, 12, 14, and 15. Five patients had heterogeneous missense mutations in exon 12 (table S2). Mutations identified at a lower frequency included missense mutations in the glycine loop encoded by exon 11 (n = 3), the catalytic domain encoded by exon 14 (n = 1), and the DFG motif in the activation segment (exon 15; n = 3). All parents and controls, totaling 40 phenotypically unaffected individuals, had none of these mutations, which supports the hypothesis that the occurrence of CFC is sporadic.

Although the causative mutations were heterogeneous, the distribution of mutations was specific and nonrandom. No frameshift, nonsense, or splice mutations were detected in the cohort of patients; thus, *BRAF* haploinsufficiency is not a likely causative mechanism of CFC. In 5 out of 23 (22% of) individuals with CFC syndrome, no *BRAF* mutations were identified. Three of these individuals were found to have missense mutations in *MEK1* (NM 002755) and *MEK2* (NM 030662) that encode downstream effectors of B-Raf (Fig. 1C). Two individuals had heterogeneous mutations in *MEK1*: nt158T→C transition (F53S) and a nt389A→G transition (Y130C) in the protein kinase domain (fig. S1). CFC patient number 21 had a *MEK2* missense nt170T→G transversion, predicting a F57C substitution (Fig. 1D). Interestingly, F57 in MEK2 (MAPK kinase 2) is the equivalent position to F53 in the closely related MEK1, which suggests that substitutions of this residue may have similar functional consequences in the two family isoforms. The disease-causing mutations in the other two individuals remain to be identified.

The Raf/MEK/ERK cascade is the best understood of the MAPK pathways. (ERK, the extracellular signal–regulated kinase, is a type of MAPK.) In addition to B-Raf, the Raf family includes C-Raf-1 and the X-linked A-Raf. The expression pattern of each isoform is distinct (*6*), and genetic studies in mice have revealed nonredundant developmental functions (*7*, *8*). Somatic mutations in *BRAF* occur at high frequency in numerous human cancers (*9*). One mutation, ^{V600E}B-Raf, which confers increased kinase activity, accounts for more than 90% of these mutations. Its presence in benign nevi, as well as primary and metastatic melanoma, suggests that MAP kinase activation is important in melanocytic neoplasia but insufficient for tumorigenesis (*10*).

In contrast to the mutation spectrum seen in cancer, the *BRAF* missense mutations identified in individuals having CFC syndrome are more widely distributed (Fig. 1A). Of the 11 different missense amino acid substitutions, only five involve codons that are altered in cancer (table S2), yet only two individuals with CFC syndrome, both of whom have severe phenotypes, have the same substitution that has been reported in cancer (fig. S2). To explore the functional consequences of these mutations, we compared the kinase activity of the CFC B-Raf mutants to that of the wild-type protein (^{WT}B-Raf) and several cancer-derived mutants in transfected human embryonic kidney 293T cells (Fig. 2A; SOM). Four of the CFC B-Raf mutants had increased kinase activity compared with ^{WT}B-Raf, and this activity was as high as that of the ^{V600E}B-Raf mutant found in cancer. Two CFC B-Raf mutants had lower activity than ^{WT}B-Raf and appear to be kinase-impaired (*11*). Thus, the type of B-Raf mutations found in

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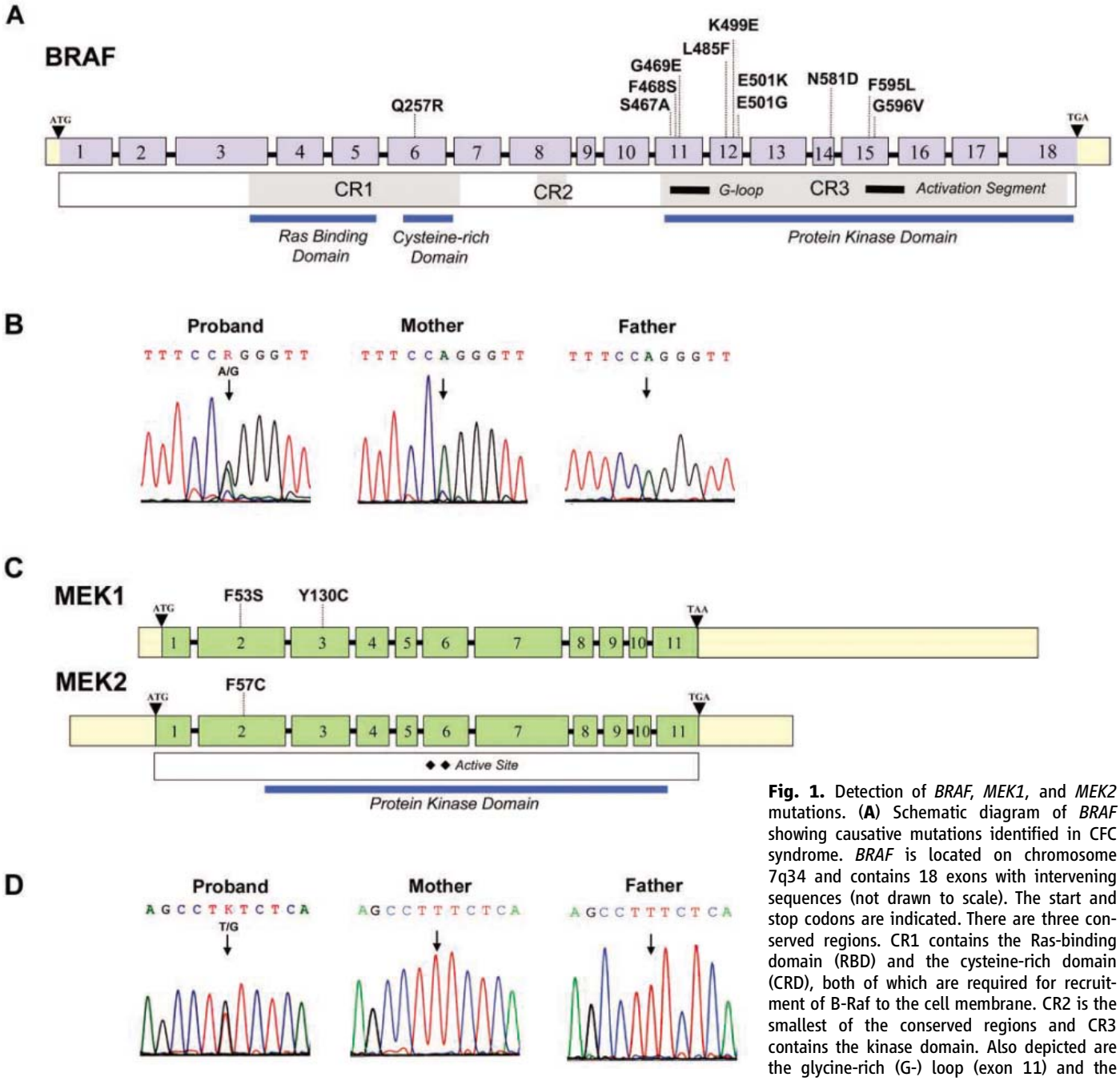


Fig. 1. Detection of *BRAF*, *MEK1*, and *MEK2* mutations. (**A**) Schematic diagram of *BRAF* showing causative mutations identified in CFC syndrome. *BRAF* is located on chromosome 7q34 and contains 18 exons with intervening sequences (not drawn to scale). The start and stop codons are indicated. There are three conserved regions. CR1 contains the Ras-binding domain (RBD) and the cysteine-rich domain (CRD), both of which are required for recruitment of B-Raf to the cell membrane. CR2 is the smallest of the conserved regions and CR3 contains the kinase domain. Also depicted are the glycine-rich (G-) loop (exon 11) and the activation segment (exon 15) of the catalytic domain. The 11 missense mutations identified in our cohort of individuals with CFC syndrome are depicted. (**B**) Lymphocyte DNA electropherograms of a proband and parents are shown identifying a *BRAF* missense mutation in exon 6 in the proband. Parental DNA samples show normal wild-type sequences. (**C**) Schematic diagram of genes *MEK1* and *MEK2* showing causative mutations identified in CFC syndrome. The *MEK1* gene is located on chromosome 15q22.31, and *MEK2* is on chromosome 19p13.3. Each gene contains 11 exons with intervening sequences (not drawn to scale). Missense mutations identified in three individuals with CFC syndrome are depicted. (**D**) Lymphocyte DNA electropherograms of the proband and parents are shown identifying the *MEK2* missense mutation in exon 2. Parental DNA samples show normal wild-type sequences.

CFC recapitulates the different types of mutations found in cancer, those with high kinase and kinase-impaired activities. To determine the ability of CFC B-Raf mutants to activate downstream effectors, we measured phosphorylated species of MEK and ERK in transfected cells by Western blotting (Fig. 2B; SOM). Both cancer- and CFC-associated B-Raf mutants with elevated kinase activity (Fig. 2A) induced higher levels of MEK and ERK phosphorylation compared with ^{WT}B-Raf, whereas kinase-impaired B-Raf mutants were impaired in their ability to induce phosphorylation of MEK and ERK (Fig. 2B).

Missense mutations in *MEK1* and *MEK2*, which encode the only known effectors of B-Raf, also cause CFC syndrome. MEK1 and MEK2 are dual-specificity kinases that both activate ERK1 and ERK2 but appear to play nonredundant roles. Genetic evidence from mouse models indicates that MEK1 is essential for embryonic development (*12*), whereas MEK2 is dispensable (*13*). Although activation of MEK is necessary for mammalian cell transformation

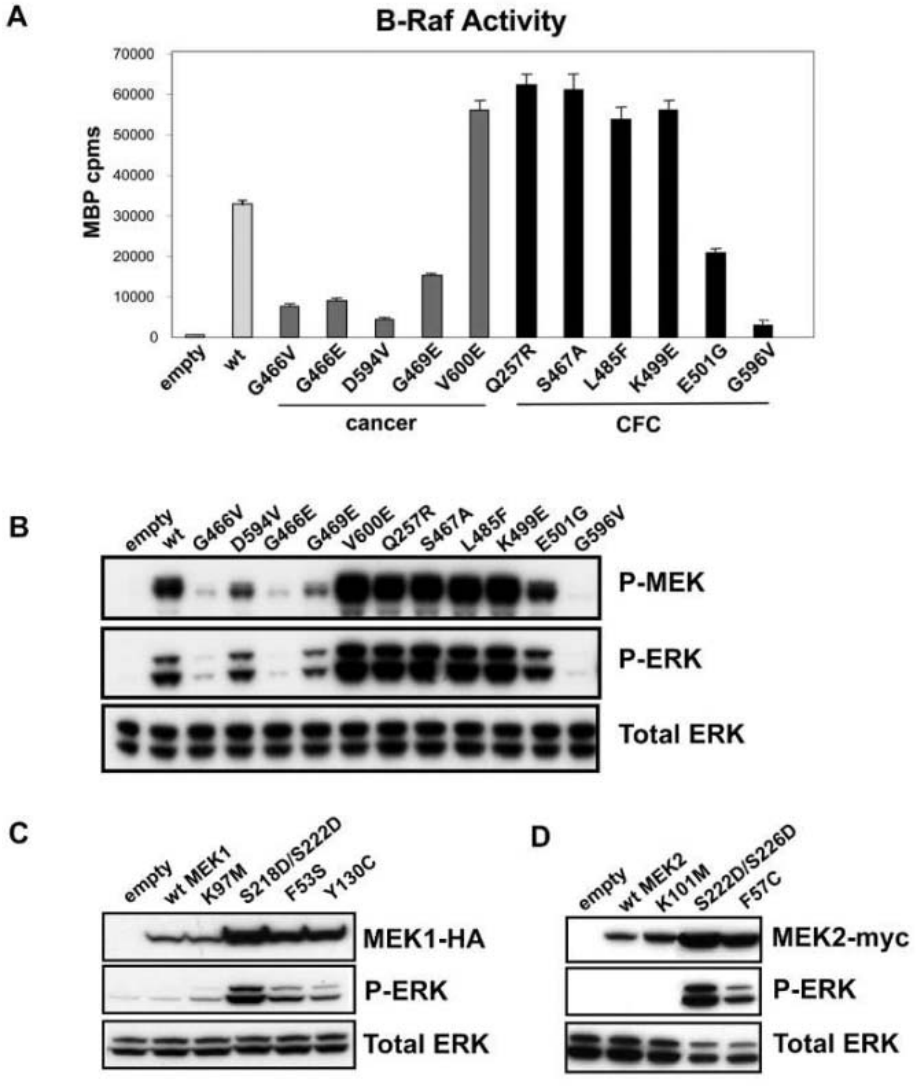


Fig. 2. Functional characterization of B-Raf and MEK mutants identified in CFC. (**A**) Kinase activities of B-Raf missense CFC mutants (black bars) are compared with those of B-Raf mutations found in cancer (gray bars). Empty vector, wild type B-Raf (^{WT}B-Raf), or the indicated B-Raf point mutants were transfected in 293T cells and B-Raf activity was measured on Flag-immunoprecipitates using a coupled MEK-ERK assay with myelin basic protein (MBP) as the final substrate. Error bars represent the standard deviation of duplicates. (**B**) MEK and ERK activation by B-Raf mutants. Lysates from 293T cells transfected with empty vector, ^{WT}B-Raf, or B-Raf mutants were subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE), and MEK and ERK (p44 ERK1 and p42 ERK2) phosphorylation levels were assayed by Western blotting using phospho-specific antibodies. Total ERK antibody staining is shown as a loading control. (**C**) ERK activation by ^{F53S}MEK1 and ^{Y130C}MEK1 CFC mutants. ERK phosphorylation induced by the CFC MEK1 mutants were compared with that induced by empty vector, ^{WT}MEK1, ^{K97M}MEK1, which is a kinase inactive mutant, and $^{S218D/S222D}$MEK1, which is a constitutively active mutant (*13*). ERK phosphorylation (p44 ERK1 and p42 ERK2) was assayed by Western blotting using phospho-specific antibodies. Hemagglutinin (HA)–tagged MEK1 is shown as a measure of transfection efficiency, and total ERK is shown as a loading control. (**D**) ERK activation by the ^{F57C}MEK2 CFC mutant. ERK phosphorylation induced by the CFC ^{F57C}MEK2 mutant is compared with that induced by empty vector, ^{WT}MEK2, ^{K101M}MEK2, which is a kinase inactive mutant, and $^{S222D/S226D}$MEK2, which is a constitutively active mutant (*13*). ERK phosphorylation (p44 ERK1 and p42 ERK2) was assayed by Western blotting using phospho-specific antibodies. Myc-tagged MEK2 is shown as a measure of transfection efficiency, and total ERK is shown as a loading control.

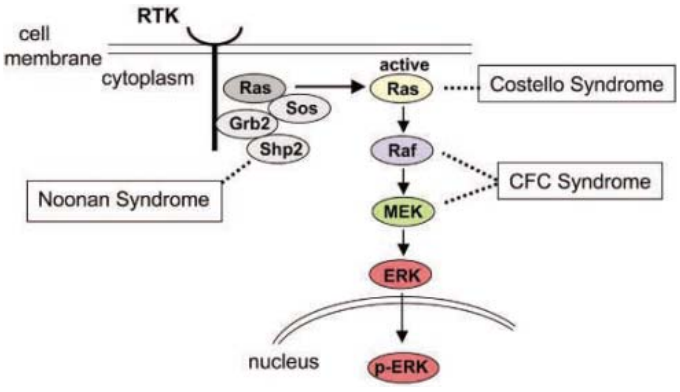
through the MAPK cascade (*14*) and constitutively active MEK mutants promote transformation (*15*), mutations in MEK1 and MEK2 have thus far not been reported in human cancer (*9*, *16*). Three individuals in the CFC cohort (13%) had de novo missense mutations, with two mutations occurring in equivalent positions within exon 2 of *MEK1* and *MEK2* (Fig. 1C). To explore the functional consequences of these substitutions, we assayed ERK phosphorylation in transfected cells by Western blotting (Fig. 2, C and D). All CFC MEK mutants, ^{F53S}MEK1, ^{Y130C}MEK1, and ^{F57C}MEK2 were more active than wild-type MEK in stimulating ERK phosphorylation, but they were not as active as the constitutively active MEK mutants. Although our current CFC cohort with MEK1/2 mutations is few in number, the phenotypic features of individuals are concordant with those observed in mouse models (fig. S1). Transgenic mice expressing activated MEK1 have enhanced MEK1-ERK1/2 signaling and exhibit compensated cardiac hypertrophy (*17*), hyperkeratosis and epidermal hyperproliferation (*18*, *19*), and cataract formation (*20*).

CFC syndrome, a developmental disorder that is phenotypically similar to NS and CS, is unique in that it is caused by missense mutations in one of three different signaling components of the Ras/MAPK pathway (Fig. 3). Interestingly, unlike NS or CS, CFC syndrome has not been considered a cancer-predisposing syndrome, because individuals do not develop malignancies. Our findings highlight the involvement of the MAPK pathway in human development. Individuals with the suspected clinical diagnosis of CFC syndrome can now be diagnosed on a molecular basis. Because the MAPK pathway has been studied intensively in the context of cancer, therapeutics that specifically target this pathway are in development. Inhibitors of Raf and MEK are being evaluated in clinical trials and appear to be well tolerated (*21*). In addition, a recent report indicates that cells with activated B-Raf have enhanced, selective sensitivity to MEK inhibitors (*22*). Because CFC has an evolving phenotype, systemic therapies that reduce MAPK activity may merit investigation in this population of patients.

References and Notes

1. M. Tartaglia *et al.*, *Nat. Genet.* **29**, 465 (2001).
2. Y. Aoki *et al.*, *Nat. Genet.* **37**, 1038 (2005).
3. A. L. Estep, W. E. Tidyman, M. A. Teitell, P. D. Cotter, K. A. Rauen, *Am. J. Med. Genet. A.* **140**, 8 (2006).
4. J. F. Reynolds *et al.*, *Am. J. Med. Genet.* **25**, 413 (1986).
5. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
6. S. M. Storm, J. L. Cleveland, U. R. Rapp, *Oncogene* **5**, 345 (1990).
7. C. A. Pritchard, L. Bolin, R. Slattery, R. Murray, M. McMahon, *Curr. Biol.* **6**, 614 (1996).
8. L. Wojnowski, L. F. Stancato, A. C. Larner, U. R. Rapp, A. Zimmer, *Mech. Dev.* **91**, 97 (2000).
9. www.sanger.ac.uk/genetics/CGP/cosmic.
10. P. M. Pollock *et al.*, *Nat. Genet.* **33**, 19 (2003).
11. P. T. Wan *et al.*, *Cell* **116**, 855 (2004).
12. S. Giroux *et al.*, *Curr. Biol.* **9**, 369 (1999).
13. L. F. Belanger *et al.*, *Mol. Cell. Biol.* **23**, 4778 (2003).
14. S. Cowley, H. Paterson, P. Kemp, C. J. Marshall, *Cell* **77**, 841 (1994).
15. S. J. Mansour *et al.*, *Science* **265**, 966 (1994).

Fig. 3. Ras/Raf/MEK/ERK signal transduction pathway and associated genetic syndromes.



16. A. Bansal, R. D. Ramirez, J. D. Minna, *Oncogene* **14**, 1231 (1997).
17. O. F. Bueno *et al.*, *EMBO J.* **19**, 6341 (2000).
18. R. M. Hobbs, V. Silva-Vargas, R. Groves, F. M. Watt, *J. Invest. Dermatol.* **123**, 503 (2004).
19. F. A. Scholl, P. A. Dumesic, P. A. Khavari, *Cancer Res.* **64**, 6035 (2004).
20. X. Gong *et al.*, *Invest. Ophthalmol. Vis. Sci.* **42**, 539 (2001).
21. J. S. Sebolt-Leopold, R. Herrera, *Nat. Rev. Cancer* **4**, 937 (2004).
22. D. B. Solit *et al.*, *Nature*, published online 6 November 2005 (10.1038/nature04304).
23. We thank CFC International, the families, and donors for their profound contributions; the Genetic Alliance BioBank; and E. Johnson, J. Carey, D. Pinkel, J. Phuchareon, P. Conger, V. St. Pierre, and S. Lee. Supported by NIH grant HD048502 and The Mt. Zion Health Fund (K.A.R.), The Concern and V Foundations (O.T.), and The Durra Foundation and Bristol-Myers Squibb (F.M.).

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Materials and Methods
Figs. S1 and S2
Tables S1 and S2
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Combined Analog and Action Potential Coding in Hippocampal Mossy Fibers

Henrik Alle and Jörg R. P. Geiger*

In the mammalian cortex, it is generally assumed that the output information of neurons is encoded in the number and the timing of action potentials. Here, we show, by using direct patch-clamp recordings from presynaptic hippocampal mossy fiber boutons, that axons transmit analog signals in addition to action potentials. Excitatory presynaptic potentials result from subthreshold dendritic synaptic inputs, which propagate several hundreds of micrometers along the axon and modulate action potential–evoked transmitter release at the mossy fiber–CA3 synapse. This combined analog and action potential coding represents an additional mechanism for information transmission in a major hippocampal pathway.

The prevailing mode to encode information in the mammalian central nervous system is to convert an analog signal resulting from graded synaptic inputs into patterns of action potentials (APs) (*1*), which are transmitted as all-or-none signals along the axons. By contrast, in primary sensory systems and central neural circuits of small invertebrates, analog signals are used directly to transmit information (*2*, *3*). Comparison of the two modes has revealed that AP coding is less efficient than analog coding at transmitting information (*4*, *5*). Because in many brain regions the axonal distances from the cell body to a large fraction of the corresponding presynaptic boutons are rather short (*6*) and somatic subthreshold signals can be large in amplitude (*7*, *8*), the question arises whether analog axonal signaling contributes to information transmission in the mammalian brain.

To study subthreshold electrical signaling in a cortical presynaptic terminal, we obtained recordings from hippocampal mossy fiber boutons (MFBs) of rats (*9*, *10*) (Fig. 1A). In hippocampal slices at a recording temperature of 34° ± 1°C, extracellular stimulation in the molecular layer of the dentate gyrus (DG-ML) resulted in slow, transient depolarizing voltage deflections at the MFB (Fig. 1B), which we termed excitatory presynaptic potentials (EPreSPs). The peak amplitudes of EPreSPs depended on stimulus intensity in a graded manner (Fig. 1C). Furthermore, EPreSPs exhibited moderate trial-to-trial peak amplitude fluctuations and a slow time course (Fig. 1D). The recorded EPreSPs had a mean peak amplitude of 4.3 ± 0.2 mV and a rise time and a half duration of 20 ± 0.7 ms and 97 ± 3 ms, respectively (n = 49). Underlying currents (EPreSCs) recorded in the voltage-clamp configuration had a peak amplitude of 3.8 ± 0.3 pA, a rise time of 12 ± 1.4 ms, a decay time constant of 46 ± 4 ms, and a half duration of 50 ± 3 ms (n = 10) (Fig. 1D). EPreSP peak amplitudes were reversibly reduced by the bath-applied ionotropic glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM) to 0.25 ± 0.02 of control peak amplitudes (n = 6) (Figs. 1E and 2D). Similarly, the selective AMPA receptor antagonist GYKI 53655 (30 μM) reversibly reduced EPreSPs to 0.11 ± 0.03 of control (n = 3) (*11*), indicating that the generation of EPreSPs required AMPA receptor activation.

EPreSPs could be generated either locally in CA3–stratum lucidum (CA3-SL), for example, by heterosynaptic activation of presynaptic ionotropic receptors (*12*, *13*), or remotely as excitatory postsynaptic potentials (EPSPs) at the granule cell dendrites followed by axonal propagation.

Local perfusion of CNQX to the CA3-SL (Fig. 2A) did not significantly reduce the EPreSP peak amplitude compared to control (Fig. 2, B and D) (n = 4, $P > 0.5$), in contrast to the effect of bath application. However, simultaneously recorded field potentials (Fig. 2B) near the MFB recording pipette were reversibly reduced (Fig. 2, B and D) (n = 4, $P < 0.05$), indicating that local drug application was sufficient to block local glutamate receptors. Similarly, local perfusion of tetrodotoxin (TTX) at the hilar end of CA3 (Fig. 2A) resulted in a reversible block of recorded field potentials (0.08 ± 0.05 of control) but attenuated EPreSP amplitudes only slightly, to 0.89 ± 0.04 of control (Fig. 2, C and E) (n = 4, $P < 0.05$). This suggests that EPreSPs were not generated locally in CA3 but represent forward-propagated EPSPs from granule cell dendrites. We consistently found in all post hoc stainings from EPreSP recordings (n = 16) that the corresponding MFB, its axon, and the granule cell soma were anatomically connected (Fig. 3A).

Nonlinear regression analysis of the maximal EPreSP peak amplitudes recorded in MFBs and the respective post hoc determined distances of the recording site to soma revealed a significant correlation (Fig. 3B) (n = 8, $P < 0.05$, and mean distance of 700 μm). Data points were fitted by using a monoexponential function $A_0 \cdot \exp(-\lambda_{EPreSP}/x)$, yielding an average somatic peak depolarization (A_0) of 22 mV and a transient-

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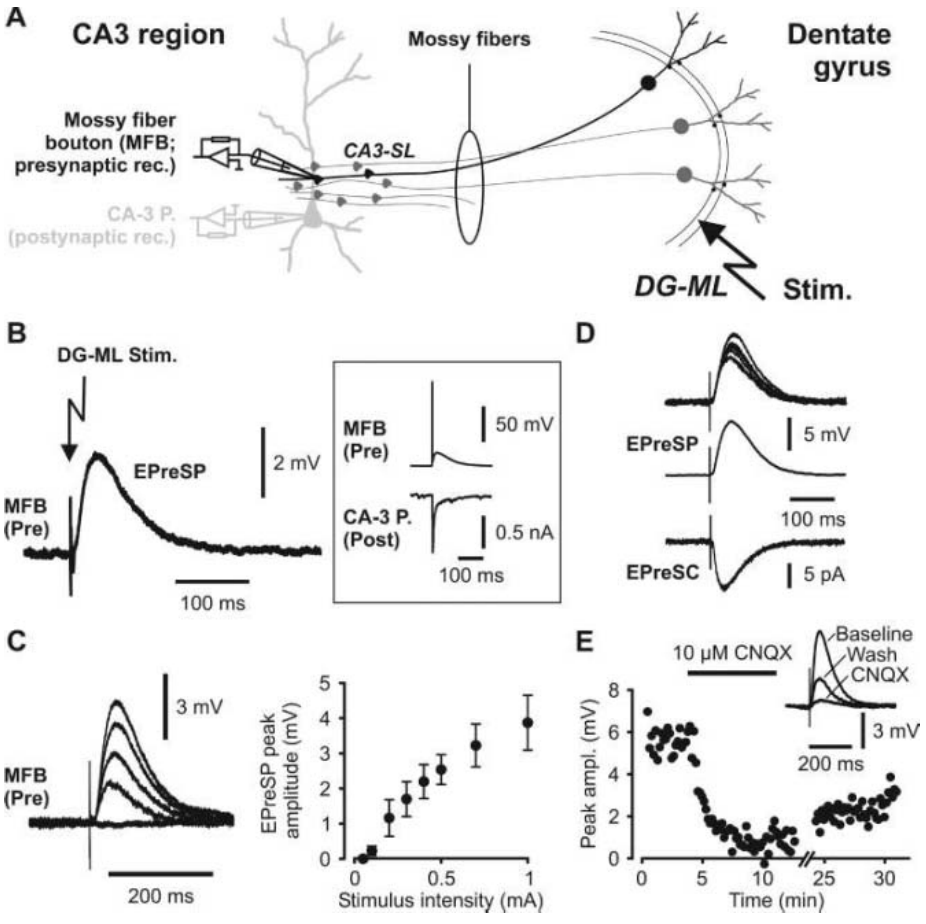


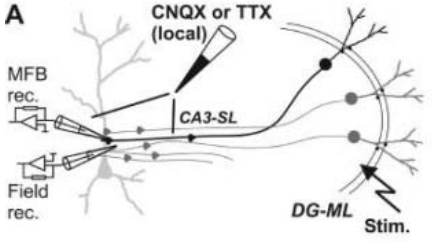
Fig. 1. EPreSPs in MFBs. (**A**) Stimulation and recording configuration. Direct presynaptic recordings from MFBs at 34°C in the CA3-SL and extracellular stimulation (arrow) in the DG-ML. (**B**) (Left) EPreSP recorded from a MFB in the CA3-SL evoked by extracellular stimulation. (Right) A simultaneous recording of the same MFB and a postsynaptic CA3–pyramidal neuron (CA3-P), indicating the functional presynaptic nature of the MFB: top shows MFB AP; bottom, unitary EPSC in CA3-P. (**C**) (Left) EPreSP peak amplitudes depend on stimulus intensity in a graded manner. (Right) Summary of four experiments. (**D**) (Top) Individual EPreSPs superimposed, illustrating the peak amplitude fluctuation of EPreSPs at a fixed stimulus intensity. (Middle) Average EPreSP. (Bottom) Corresponding average EPreSC recorded in the voltage-clamp configuration. (**E**) Reversible reduction of EPreSP amplitude by AMPA and kainate antagonist CNQX (10 μM, bath application). (Inset) Corresponding average EPreSPs.

signal space constant (λ_{EPreSP}) of 430 μm. EPSPs of comparable amplitude (up to 35 mV) were recorded from granule cell somata upon stimulation in DG-ML (fig. S1) because granule cells in the dentate gyrus exhibit negative resting potentials close to –80 mV (fig. S1) (n = 16). Similar EPSP amplitudes and resting potentials were reported both in vitro (*14*, *15*) and in vivo (*16*, *17*).

To relate amplitude and shape of somatic EPSPs and EPreSPs, we developed a passive compartmental model of a schematized granule cell including axon and MFBs based on realistic morphology (*10*) and passive membrane properties (fig. S2). The model reproduced experimentally determined parameters like membrane time constant τ_0 (62 ± 4 ms, n = 10) (Fig. 3C), mossy fiber input resistance (1.4 ± 0.1 GΩ, n = 31), and MFB capacitance (*9*, *18*).

The constrained model reproduced the experimentally determined distance dependence of EPreSP peak amplitude, yielding a λ_{EPreSP} of about 450 μm. The axonal steady state space constant from model simulations was estimated to be around 700 μm (fig. S2), which is larger than expected from previous mossy fiber modeling (*19*) but very similar to values found in the posterior pituitary (*20*).

Simulated somatic EPSPs and measured granule cell EPSPs had half durations of 39 ms and 36 ± 2 ms (n = 10), respectively (figs. S1 and S2). Simulated EPreSPs (at 750 μm) and measured EPreSPs (mean distance to the soma of 700 μm) had half durations of 84 ms and 99 ± 10 ms (n = 8), respectively (Fig. 1 and fig. S2). Thus, the slow time course of EPreSPs can be explained by axonal filtering during passive propagation.



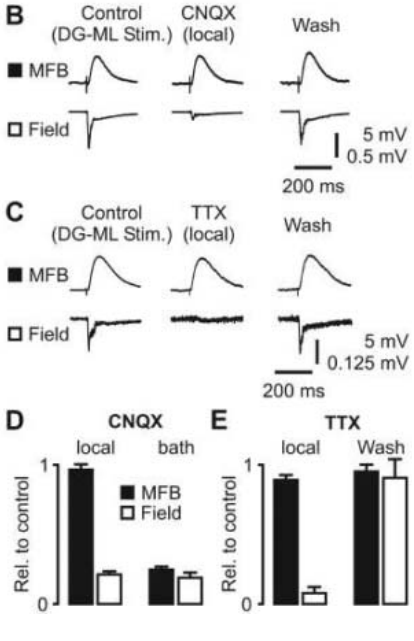


Fig. 2. EPreSPs are generated upstream of the CA3 region. (**A**) Scheme illustrating local perfusion of CNQX or TTX in CA3-SL. (**B** and **D**) Differential effect of locally and bath-applied CNQX on peak amplitudes of EPreSPs (MFB, solid) and simultaneously recorded field potentials (open) (n = 4). Field potentials in CA3-SL were recorded near (50 to 100 μm) the MFB recording pipette. (**C** and **E**) EPreSPs were only slightly attenuated but not blocked by local TTX application (1 μM) in CA3-SL; in contrast, simultaneously recorded field potentials were reversibly blocked (n = 4). Stimulation artefacts blanked in field recordings. Error bars in (D) and (E) indicate ± SEM.

To prove experimentally that depolarization of granule cell dendrites is sufficient to substantially depolarize MFBs in CA3-SL, we puff-

applied focally 1 mM RS-AMPA in the DG-ML after blocking synaptic transmission (Fig. 3, A and D). The average AMPA-mediated depolarization at MFBs was 7.7 ± 0.4 mV (n = 5) and, in the presence of TTX (0.5 μM), 6.1 ± 0.8 mV (n = 4) (Fig. 3D; somatic responses, fig. S1), demonstrating passive propagation of subthreshold depolarizations to MFBs (Fig. 3D).

The propagation of depolarizing signals raises the question of whether hyperpolarizing signals also propagate. The negative resting membrane potential of granule cells does not favor the generation of large-amplitude hyperpolarizations at the soma under the experimental conditions used. However, it is conceivable that hyperpolarizing potentials propagate when generated by inhibitory inputs during depolarized membrane potential states, as observed in vivo (*7*).

To address the functional importance of transient subthreshold depolarizations, we first studied the interaction of EPreSPs and presynaptic APs. APs recorded in MFBs were elicited by stimulation in DG-ML. A comparison of APs in isolation with APs of an EPreSP-AP combination, which was evoked by a double stimulation (at subthreshold and suprathreshold intensity, separated by 50 ms), did not reveal a detectable difference in AP shape (n = 4) (Fig. 4, A and C). Even steady state depolarizations of comparable amplitude caused only little changes in AP shape (fig. S3). Second, to test whether EPreSPs change presynaptic calcium signaling, we performed presynaptic voltage-clamp experiments to analyze the presynaptic calcium current underlying waveforms of an AP, an EPreSP, and a combination of both. Consistent with the properties of presynaptic calcium channels in MFBs (*21*), no calcium current was detectable during EPreSP waveforms, and calcium transients did not differ between waveforms of the AP and those of the combination of EPreSP and AP (n = 6) (Fig. 4, B and C). Third, we compared three presynaptic conditions in paired recordings from MFBs and postsynaptic CA3 neurons. In contrast to APs, EPreSPs (evoked by local injection of an EPreSC waveform) did not elicit a detectable signal in the postsynaptic neuron (Fig. 4D), but the combination of EPreSPs and APs (APs 10 to 20 ms after the peak of the EPreSPs) evoked markedly larger average excitatory postsynaptic currents (EPSCs) in the postsynaptic neuron than did APs alone (1.43 ± 0.09 of unconditioned trials, n = 11, $P < 0.005$) (Fig. 4, D and E), indicating that EPreSPs enhance AP-evoked transmitter release. The EPreSP enhancement of EPSCs was less pronounced when APs were timed in the late decay phase or in the late period of a nondecaying EPreSP (fig. S4).

In paired recordings using 10 mM of the calcium chelator EGTA in the presynaptic recording solution, we still found EPreSP enhancement of EPSCs (1.21 ± 0.04, n = 5, $P <$ 0.05) (Fig. 4E), but the extent was attenuated (10 mM EGTA versus control, P = 0.06). Therefore, the extent of EPreSP enhancement of EPSCs seems to depend on background calcium signaling, but our results are also consistent with a partial direct voltage modulation of release machinery, suggesting a different transduction mechanism of subthreshold signals at the MFB than that found at the calyx of Held (*22*).

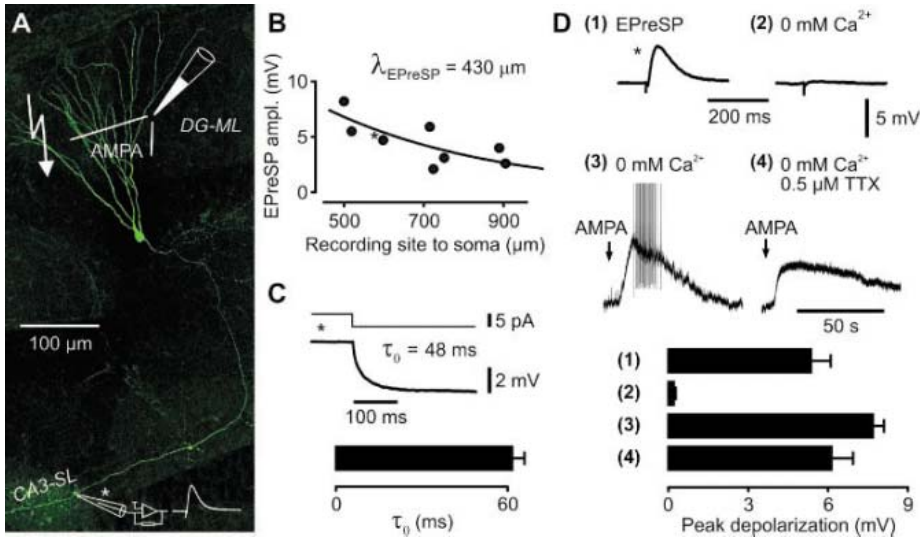


Fig. 3. Propagation of somatic depolarizations underlies EPreSPs. (**A**) Post hoc confocal image of a granule cell filled with biocytin during a MFB recording. Schematic patch pipette indicates recording site. Schematic pipette in DG-ML indicates site of AMPA puff application. (**B**) Maximal EPreSP peak amplitude of individual experiments correlated with distance of recording site to granule cell soma ($P < 0.05$, n = 8); monoexponential fit curve superimposed. (**C**) Mossy fiber steady state membrane τ_0 was estimated from hyperpolarizing pulses (for bar graph, n = 10). (**D**) Propagation of dendritic depolarization to MFBs. Step 1: EPreSP evoked in a MFB. Step 2: Block of synaptic transmission (modified artificial cerebrospinal fluid: 0 mM Ca^{2+}, 3 mM Mg^{2+}). Step 3: Focal AMPA puff application in DG-ML evoked a transient depolarization at the MFB in combination with APs (see fig. S1 for somatic AMPA puff responses). Step 4: Additional TTX (0.5 μM) abolished APs. Bars in the lower part summarize the mean peak depolarizations at the MFBs caused by the sequence of conditions 1 to 4 shown in the upper part of (D) (n either 4 or 5). Asterisks in (A) to (D), traces 1 to 4, mark measurements of the same MFB recording. Error bars, ± SEM.

In vivo, large amplitude theta oscillations (20 mV) in combination with APs have been described in granule cells (*7*). Subthreshold oscillations of such amplitude are likely to be propagated to presynaptic terminals (fig. S2). Therefore, we compared postsynaptic responses to APs (three APs at 5 Hz) with and without presynaptic subthreshold theta-like oscillations (5 Hz) in paired recordings (Fig. 4, F and G). Presynaptic subthreshold theta oscillations enhanced EPSCs while preserving multiple pulse facilitation (*23*) (Fig. 4G). This suggests a general functional role of subthreshold membrane potential changes to the regulation of synaptic transmission during naturally occurring activity patterns of granule cells (*24*). A similar function of subthreshold oscillations for synaptic output was found in invertebrates (*25*, *26*).

In conclusion, the output information of hippocampal granule cells is not exclusively encoded in the number and timing of APs. The combined analog and AP coding reported here is likely to enhance information capacity of synapses and may increase the computational power of the dentate gyrus–CA3 network.

References and Notes

1. F. Rieke, D. Warland, R. De Ruyter van Steveninck, W. Bialek, *Spikes: Exploring the Neuronal Code* (Bradford MIT Press, Cambridge, MA, 1998).
2. V. M. Pasztor, B. M. H. Bush, *Science* **215**, 1635 (1982).
3. M. Juusola, A. S. French, R. O. Uusitalo, M. Weckström, *Trends Neurosci.* **19**, 292 (1996).
4. R. R. De Ruyter van Steveninck, S. B. Laughlin, *Nature* **379**, 642 (1996).
5. M. Juusola, A. S. French, *Neuron* **18**, 959 (1997).
6. R. J. Douglas, C. Koch, M. Mahowald, K. A. C. Martin, H. H. Suarez, *Science* **269**, 981 (1995).
7. M. D. Munoz, A. Nunez, E. Garcia-Austt, *Brain Res.* **509**, 91 (1990).
8. C. C. H. Petersen, T. T. G. Hahn, M. Mehta, A. Grinvald, B. Sakmann, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13638 (2003).
9. J. R. P. Geiger, P. Jonas, *Neuron* **28**, 927 (2000).
10. L. Acsady, A. Kamondi, A. Sik, T. Freund, G. Buzsaki, *J. Neurosci.* **18**, 3386 (1998).
11. H. Alle, J. R. P. Geiger, unpublished data.
12. D. Schmitz, J. Mellor, R. A. Nicoll, *Science* **291**, 1972 (2001); published online 8 February 2001 (10.1126/science.1057105).
13. A. Ruiz *et al.*, *Neuron* **39**, 961 (2003).
14. K. J. Staley, T. S. Otis, I. Mody, *J. Neurophysiol.* **67**, 1346 (1992).
15. C. Schmidt-Hieber, P. Jonas, J. Bischofberger, *Nature* **429**, 184 (2004).
16. M. Penttonen, A. Kamondi, A. Sik, L. Acsady, G. Buzsaki, *Hippocampus* **7**, 437 (1997).
17. T. M. Gao, E. M. Howard, Z. C. Xu, *J. Neurophysiol.* **80**, 2860 (1998).
18. S. Hallermann, C. Pawlu, P. Jonas, M. Heckmann, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8975 (2003).

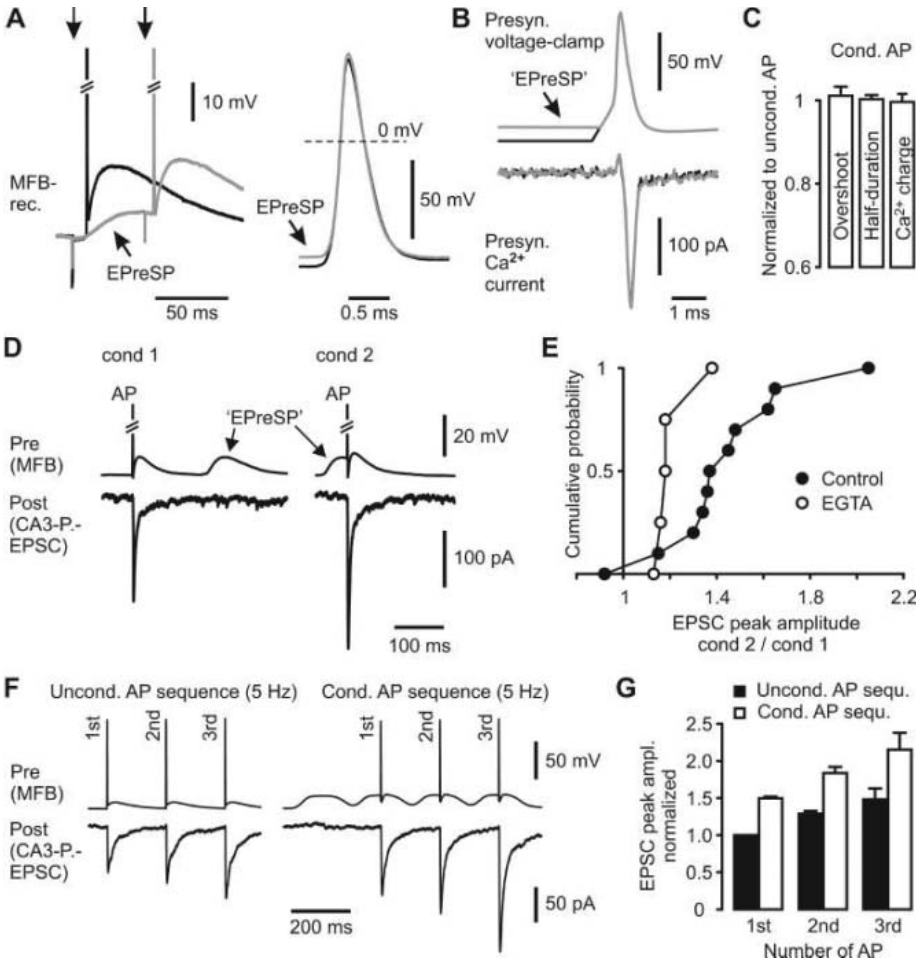


Fig. 4. EPreSPs enhance AP-evoked transmitter release. (**A**) MFB recording and stimulation in DG-ML (arrows). (Left) An AP (black trace) was elicited by suprathreshold stimulation; an EPreSP-AP sequence (gray trace) was evoked by a subthreshold followed by a suprathreshold stimulation (50-ms interval). (Right) APs superimposed. (**B**) (Top) AP and EPreSP-AP waveforms used as voltage commands in MFBs. (Bottom) Evoked presynaptic calcium currents. (**C**) Summary bar graph of experiments in (A) (n = 4) and (B) (n = 6). (**D**) Paired recordings (both AP and EPreSP were evoked by current injection into the MFB). The MFB-AP (top traces) was alternately elicited before the EPreSP (cond 1) or briefly after the peak of the EPreSP (cond 2). Bottom traces, average unitary EPSCs. (**E**) Cumulative distribution of EPSC peak amplitude ratio (cond 2/cond 1). For controls, n = 11; for 10 mM EGTA in the presynaptic pipette, n = 5. (**F**) Subthreshold theta-like oscillations modify synaptic transmission. Upper traces: left, presynaptic unconditioned AP sequence (5 Hz); right, AP sequence conditioned by theta-like oscillations. Bottom traces, average EPSCs. (**G**) Summary bar graph of experiments in (F) (n = 4). All EPSCs were normalized to the first EPSC of the unconditioned AP sequence. Error bars, ± SEM.

19. D. Engel, P. Jonas, *Neuron* **45**, 405 (2005).
20. M. B. Jackson, *J. Neurophysiol.* **69**, 692 (1993).
21. J. Bischofberger, J. R. P. Geiger, P. Jonas, *J. Neurosci.* **22**, 10593 (2002).
22. G. B. Awatramani, G. D. Price, L. O. Trussell, *Neuron* **48**, 109 (2005).
23. P. A. Salin, M. Scanziani, R. C. Malenka, R. A. Nicoll, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13304 (1996).
24. W. E. Skaggs, B. L. McNaughton, M. A. Wilson, C. A. Barnes, *Hippocampus* **6**, 149 (1996).
25. A. I. Ivanov, R. L. Calabrese, *J. Neurosci.* **23**, 1206 (2003).
26. P. S. Katz, *Curr. Biol.* **13**, R554 (2003).

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Figs. S1 to S4

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Chemical Rescue of a Mutant Enzyme in Living Cells

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The restoration of catalytic activity to mutant enzymes by small molecules is well established for in vitro systems. Here, we show that the protein tyrosine kinase Src arginine-388→alanine (R388A) mutant can be rescued in live cells with the use of the small molecule imidazole. Cellular rescue of a viral Src homolog was rapid and reversible and conferred predicted oncogenic properties. Using chemical rescue in combination with mass spectrometry, we confirmed six known Src kinase substrates and identified several new protein targets. Chemical rescue data suggest that cellular Src is active under basal conditions. Rescue of R388A cellular Src provided insights into the mitogen-activated protein kinase pathway. This chemical rescue approach will likely have many applications in cell signaling.

Elegant studies have established the utility of chemical complementation in the analysis of ligand-receptor and enzyme-inhibitor interactions in cellular systems (*1*–*3*). These approaches allow highly specific and precise temporal control of cellular pathways. Complementation of enzymes containing active-site mutations has involved functional substitution of the missing side chain with a small compound that possesses similar electronic or steric features (*4*–*6*). The obstacles to in vivo application include the requirement for a cell-permeable and relatively nontoxic small-molecule rescue agent. It was shown recently that when Arg^{318} in Csk (a conserved residue in tyrosine kinases) was mutated to Ala, it could be effectively rescued by imidazole (*7*). An actin stress fiber assay in fibroblasts suggested that R318A Csk might be rescuable in living cells (*8*). However, the maximal level of rescue was 5% of wild-type activity and this together with incomplete cellular characterization led to uncertainty in these findings.

We investigated chemical rescue with the nonreceptor protein tyrosine kinase Src. Src, the

first proto-oncogene and tyrosine kinase discovered, is involved in a myriad of cellular processes, but many aspects of its function are still poorly understood (*9*, *10*). In Src and its eight other family members, the catalytic Arg^{388} is located two residues upstream relative to its position in other tyrosine kinases (fig. S1). We therefore generated a set of Src Arg^{388} mutant recombinant proteins and examined their potential for chemical rescue (*11*) (fig. S1). The maximal turnover rate (k_{cat}) of R388A Src was about 0.5% of the k_{cat} of wild-type Src but increased to 30 to 50% of the k_{cat} of wild-type Src with imidazole. The rescued enzyme showed less than threefold changes in the Michaelis-Menten constant (K_m) of two different substrates (*11*) (fig. S1B). Encouragingly, the K_m for imidazole was ~2.5 mM (fig. S3B), well below its toxic level (*12*).

To assess the potential of chemical rescue of R388A Src kinase activity in living cells, we stably transfected R388A/Tyr^{527}→Phe (Y527F) Src in SYF (Src, Yes, and Fyn deleted) mouse embryonic fibroblasts (*13*) to generate 8A7F cells. The Y527F mutation was included to prevent down-regulation by Csk phosphorylation (*9*, *10*) and the SYF line was used because of its low background of Src family member expression. We also generated the control SYF line containing Src Asp^{386}→Asn (D386N)/Y527F (6N7F), a catalytically defective form of Src that we showed (with the use of recombinant protein) to be insensitive to imidazole (fig. S3C). Western blots with an antibody to Src showed that the level of Src expression in these transfected lines was similar to that in human embryonic kidney (HEK) 293 cells (fig. S4A). 8A7F cells were exposed to 5 mM imidazole for 0 to 10 min, and then lysates were analyzed by Western blot with a sequence nonselective antibody to phosphotyrosine (4G10) (Fig. 1A). A variety of bands intensify or freshly appear at 2.5 min and plateau by 10 min. In contrast, when the control cell line 6N7F was exposed to imidazole, no detectable changes in antiphosphotyrosine blotting were observed (Fig. 1B). That the bands seen in imidazole-treated 8A7F cells were related to rescued Src tyrosine kinase activity was also supported by the finding that pretreatment with the Src-selective inhibitor PP1 (*14*) blocked the appearance of the tyrosine-phosphorylated proteins (fig. S4B).

A more detailed analysis of the dose and reversibility of Src chemical rescue involved examining autophosphorylation on its activation-loop tyrosine, Tyr^{416} (*9*, *10*), with a site-specific antibody (Fig. 1, C and D). As expected, modification of this loop is rapid and readily detected after 5 min of imidazole treatment. About 50% maximal phosphorylation was achieved with ~2 mM imidazole exposure with 8A7F cells (Fig. 1C). Despite the more complex environment of the cellular milieu, this dose-response correlation of imidazole-Src rescue is in good agreement with that of the in vitro kinase behavior. Control 6N7F cells were insensitive to imidazole, as expected (Fig. 1C). Washout experiments showed that activation-loop phosphorylation was back to background within 4 min of imidazole removal (Fig. 1D). This experiment not only establishes the rapid reversibility of kinase activity by dilution of imidazole, but it also underscores the rapid cellular kinetics of tyrosine phosphatase activity toward the Src activation loop.

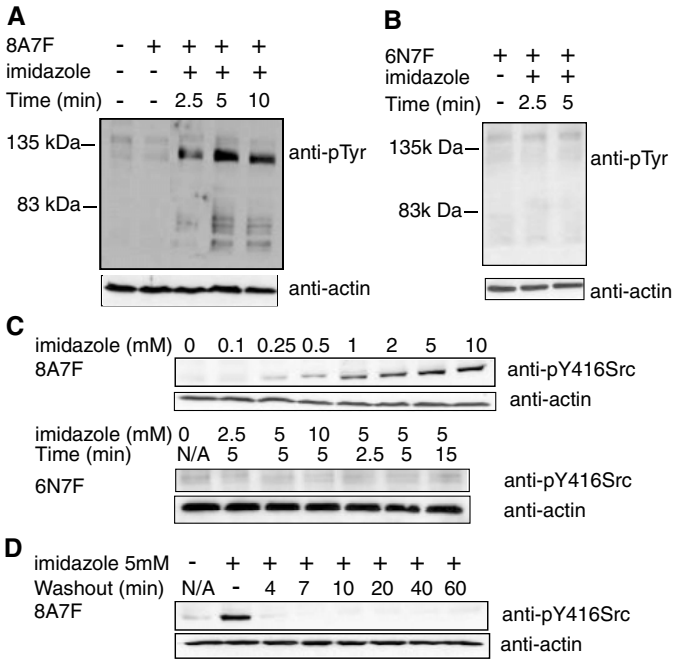

Fig. 1. Tyrosine phosphorylation induced by imidazole treatment of Src R388A/Y527F in SYF cells. (**A** and **B**) Time course of phosphorylation levels in 8A7F cells (A) and 6N7F cells (B) treated with 5 mM imidazole. Lysates were analyzed by blot with a 4G10 antibody. (**C**) Src autophosphorylation (Y416) in 8A7F and 6N7F cells as a function of imidazole concentration. 8A7F cells received 5 min of treatment; 6N7F cells were treated with the imidazole concentrations and times shown. (**D**) Reversible autophosphorylation after imidazole washout. 8A7F cells were treated with 5 mM imidazole (5 min), and then imidazole-containing media was exchanged for imidazole-free media, and lysates were analyzed 4 to 60 min later.

To further analyze the scope and kinetics of the chemical rescue process in 8A7F cells, we transiently transfected 8A7F cells with a fluorescent biosensor that shows a fluorescence resonance energy transfer (FRET) change upon Src phosphorylation (*15*) (Fig. 2A). Imidazole induces a rapid (less than 60 s) FRET change in cytoplasmic regions of the cell. The FRET change plateaus by about 500 s, consistent with an equilibrium being reached with phosphatase action and also with the effects seen by Western blot (Fig. 1). Pretreatment with the Src-selective inhibitor PP1 abolished FRET change (Fig. 2A).

Functional and longer term consequences of chemical rescue were also studied with the use of a cellular transformation assay (*16*) as well as a matrigel invasion analysis (*17*) (Fig. 2). Addition of imidazole to 8A7F cells but not 6N7F cells led to increases in the number of transformed foci (Fig. 2B and fig. S4C) and also enhanced the efficiency of invasion with the use of a modified Boyden chamber assay (Fig. 2C). These classical phenotypes associated with viral Src (v-Src)–carrying fibroblasts suggest that chemical rescue of mutant Src in cells can induce expected oncogenic properties.

To gain insight into the nature of the proteins that are tyrosine phosphorylated by imidazole after treatment of 8A7F cells, we carried out an affinity-based enrichment of tyrosine-phosphorylated proteins using antibodies to phosphotyrosine (*18*). Immunoprecipitated proteins were separated on an SDS–polyacrylamide gel electrophoresis and detected with silver staining, and bands that appeared to be enriched were identified by tandem mass spectrometry (Fig. 3A). We also used stable isotope labeling (SILAC) (*18*), which allows for relative quantitation to identify those proteins whose tyrosine-phosphorylation status is up-regulated upon imidazole treatment (Fig. 3A and fig. S5). Of the 24 proteins detected from either of the two strategies, six are reported to be Src substrates (*19*, *20*), but the others had not previously been linked to Src kinase action.

To validate the mass spectrometry results, eight individual proteins were immunoprecipitated and then Western blotted with the antibody to phosphotyrosine (Fig. 3B). Western blots of the proteins with type-specific antibodies were used for loading controls. These eight proteins reproducibly increased their phosphotyrosine content after imidazole treatment of 8A7F cells but not 6N7F control cells (Fig. 3B). Included among these were five established Src sub-

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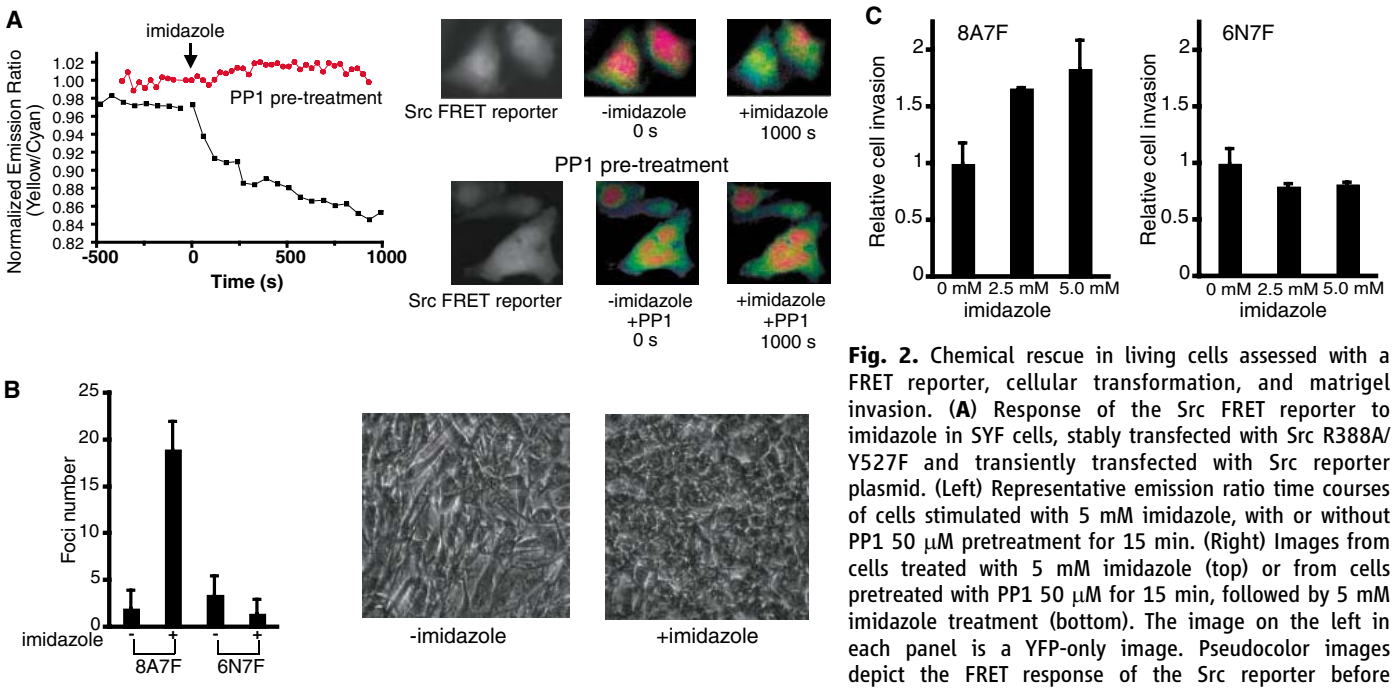

Fig. 2. Chemical rescue in living cells assessed with a FRET reporter, cellular transformation, and matrigel invasion. (**A**) Response of the Src FRET reporter to imidazole in SYF cells, stably transfected with Src R388A/Y527F and transiently transfected with Src reporter plasmid. (Left) Representative emission ratio time courses of cells stimulated with 5 mM imidazole, with or without PP1 50 μM pretreatment for 15 min. (Right) Images from cells treated with 5 mM imidazole (top) or from cells pretreated with PP1 50 μM for 15 min, followed by 5 mM imidazole treatment (bottom). The image on the left in each panel is a YFP-only image. Pseudocolor images depict the FRET response of the Src reporter before (middle image) and after (right image) imidazole treatment. (**B**) Imidazole induces focus formation in 8A7F cells. 8A7F cells or 6N7F cells were mixed with normal NIH3T3 cells and plated in 10-cm dishes, in media supplemented with or without 1 mM imidazole and grown for 15 to 19 days (*11*). The bar graph shows the number of methylene blue stained foci + standard error in duplicate 10-cm dishes. Representative phase-contrast microscopic images of transformed cells from foci in the imidazole-treated cells versus control cells. (**C**) Imidazole induces 8A7F cell invasion. We performed cell invasion assays in which cells invade over 48 hours through a modified Boyden chamber with a filter of 8-μm pore size coated with a reconstituted basement membrane matrix (*11*). Bar graph values shown contain standard errors associated with duplicate measurements.

strates [DOK1, focal adhesion kinase (FAK), paxillin, PI3Kp85, and p130-cas, not including Src itself (Fig. 1C)] as well as three substrates not previously linked to Src-mediated phosphorylation (CrkL, lamin A/C, and procollagen). CrkL is a particularly intriguing Src target because of its well-established role in cytoskeletal signaling and its known Src connections (*21*, *22*).

To further probe the physiologic importance of CrkL phosphorylation, we analyzed the potential for CrkL modification in response to epidermal growth factor (EGF) treatment of two mammalian cell types [HEK 293 cells and rat embryo fibroblast (REF) 52 cells]. EGF and other growth factors [such as platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF)] are known to be able to activate Src through their cognate receptor tyrosine kinases. Indeed, EGF treatment of these mammalian cell lines induced tyrosine phosphorylation of CrkL, and this could be blocked by treatment with a Src-selective small-molecule inhibitor (fig. S4D). These data support the notion that CrkL phosphorylation as a consequence of Src activation is likely to be of biological relevance.

We were also interested in examining the impact of Src rescue on the kinetics of gene expression. Chronic gene expression changes in v-Src transformed colon cancer and NIH3T3 cells have been reported (*23*, *24*), but the chemical rescue method permits insights into rapid

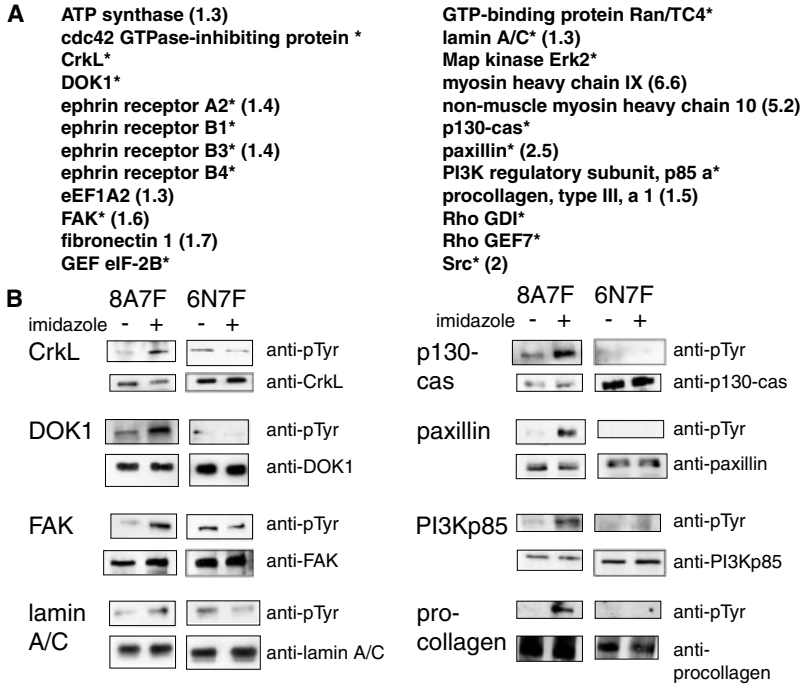

Fig. 3. Mass spectrometric analysis of tyrosine-phosphorylated proteins of chemically rescued R388A/Y527F Src and validation by immunoprecipitation-immunoblot analysis. (**A**) Proteins identified after 5 mM imidazole (5 min) treatment of 8A7F cells by mass spectrometry in two separate experiments. Stars indicate proteins identified after 4G10 antibody enrichment followed by liquid chromatography tandem mass spectrometry. SILAC analysis identified an overlapping but nonidentical set of proteins; values shown in parentheses refer to isotopic enhancement by SILAC analysis. (**B**) Immunoprecipitation-immunoblot analysis of 8A7F and 6N7F cells after treatment with 5 mM imidazole for 5 min. Immunoprecipitation was carried out with commercially available antibodies corresponding to the protein of interest and immunoblotting was carried out with the 4G10 antibody as well as type-specific antibodies.

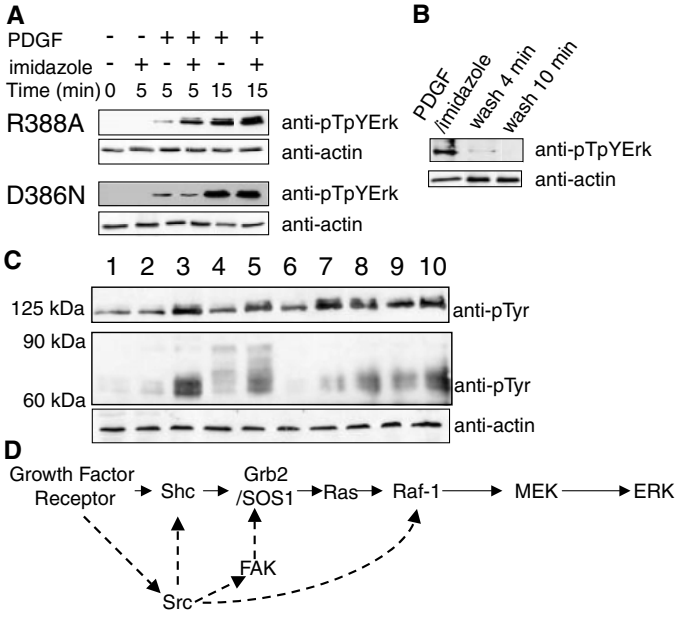

Fig. 4. Interaction of growth factors and chemical rescue of R388A c-Src on MAPK activation (anti-pTpYErk) and tyrosine phosphorylation. (**A**) Erk activation in 8A or 6N cells treated with PDGF (50 ng/ml) and/or imidazole (5 mM) from 0 to 15 min. (**B**) Erk activation requires concomitant Src and Erk activity at 5 min. 8A cells were treated with PDGF (50 ng/ml) and imidazole (5 mM), simultaneously (PDGF/imidazole); or initially with 5 mM imidazole for 5 min and then incubated in imidazole-free media for 4 min (wash 4 min) or 10 min (wash 10 min), followed by PDGF treatment (50 ng/ml) for 5 min. (**C**) Tyrosine-phosphorylation levels in 8A cells in response to growth factors and/or imidazole analyzed with the 4G10 antibody. Lane 1, control; lane 2, PDGF (50 ng/ml, 5 min); lane 3, PDGF (50 ng/ml) and imidazole (5 mM), simultaneously for 5 min; lane 4, FGF (2.5 ng/ml, 2.5 min); lane 5, FGF (2.5 ng/ml) and imidazole (5 mM), simultaneously for 2.5 min; lane 6, EGF (5 ng/ml, 2.5 min); lane 7, EGF (5 ng/ml) and imidazole (5 mM), simultaneously for 2.5 min; lane 8, imidazole (5 mM, 2.5 min); lane 9, imidazole (5 mM, 5 min); lane 10, imidazole (10 mM, 10 min). (**D**) Scheme for the role of Src in potentiating the MAPK cascade induced by growth factor engagement, indicating possible roles for Shc, FAK, and Raf-1 phosphorylation. MEK, MAPK kinase.

kinetic changes. We used gene microarray analysis of imidazole-activated Src, 1 hour after imidazole treatment of 8A7F cells and 6N7F control cells. At 1 hour, 55 genes show increases (>1.7-fold) in the 8A7F cells and another 31 show decreases (>1.7-fold) with minimal changes in 6N7F control cells (fig. S6). We further analyzed 13 of these genes using real-time reverse transcription polymerase chain reaction (fig. S6) and found that most of the genes tested showed changes that were similar across techniques. These gene changes were not reported in cells chronically transformed with v-Src (*23*, *24*) or rapidly stimulated with growth factors (*25*), suggesting that rapid initiation of Src-mediated tyrosine phosphorylation may induce a specialized pattern of gene expression changes. However, these earlier experiments were done under different conditions, which may also contribute to gene effects.

The cellular form of Src (c-Src) is a proto-oncogene maintained in a repressed catalytic state resulting from Csk phosphorylation of Tyr^{527} (*9*, *10*). Csk-treated recombinant R388A Src protein was C-terminally phosphorylated and, under conditions of imidazole rescue, showed diminished kinase activity compared with R388A Src that was not exposed to Csk (fig. S7). This indicates that chemically rescued R388A Src is still subject to the physiologic regulation mechanism of the wild-type enzyme. We generated the corresponding stably transfected R388A/SYF (8A) cell line along with the D386N/SYF (6N) control line (fig. S7). Using an antibody specific to 527-pTyr, we showed that C-terminal phosphorylation was also similar in 8A cells and HEK 293 cells, suggesting that R388A c-Src (fig. S7) would be subject to the same level of Csk-mediated suppression as wild-type c-Src.

A role for c-Src kinase activity as an effector of growth factor receptor stimulation in mitogen-activated protein kinase (MAPK) activation is controversial (*26*–*29*). Models for the involvement of FAK, Shc, and Raf1 phosphorylation have been described (Fig. 4D). We undertook an investigation of the effects of c-Src chemical rescue on MAPK activation in response to PDGF, EGF, and FGF. Using antibodies to phosphothreonine phosphotyrosine (pTpY)–Erk as a marker of MAPK activation, we demonstrated a role for c-Src kinase activity for rapid induction of MAPK signaling (Fig. 4A and fig. S7, E and F). Imidazole alone showed no ability to induce MAPK activation (Fig. 4A). With PDGF stimulation, a reproducibly enhanced MAPK activation was observed at 5 min with imidazole-treated 8A cells. In contrast, after 15 min of PDGF exposure, little difference was observed between imidazole-treated and imidazole-untreated 8A cells (Fig. 4A). Imidazole showed no effects with 6N cells, indicating that imidazole was most likely acting through c-Src kinase rescue (Fig. 4A). Related behavior was observed with EGF and FGF signaling (fig. S7, E and F). If 8A cells were treated with imidazole for 5 min, and then the imidazole was removed 4 min before PDGF stimulation, MAPK activation was not observed (Fig. 4B). Thus, Src kinase activity and PDGF stimulation must be temporally concerted to induce rapid MAPK activation. These results extend earlier findings about a role for Src in PDGF-induced MAPK activation in SYF cells (*13*).

Unexpectedly, imidazole treatment in the absence of growth factors led to the appearance of a number of bands visualizable with an antibody to phosphotyrosine within 2.5 to 10 min in 8A cells (Fig. 4C) but not 6N cells (fig. S7H). Two of the phosphorylated proteins in 8A cells are likely paxillin and FAK (fig. S7G). As mentioned, R388A c-Src shows normal apparent Csk regulation (fig. S7). These results indicate that c-Src under "basal" cellular conditions shows significant tyrosine kinase activity. PDGF, FGF, and EGF without imidazole (Fig. 4C) appeared to stimulate a distinctive but overlapping set of phosphotyrosine bands compared with imidazole alone. The combined effects of growth factors and chemical rescue (Fig. 4C) appeared to be partially additive. These results provide a framework for elucidating Src's role in MAPK pathways (Fig. 4D).

The chief advantage of chemical rescue for signaling pathway analysis rests in the ability to achieve rapid and reversible activity stimulation compared with longer term experiments with inducible transcriptional systems or temperature-sensitive mutants (*29*). Chemical rescue can provide unique signaling information compared with kinase inhibitors, because it is a positive rather than negative stimulation (*3*). Given that the catalytic loop Arg rescued is absolutely conserved in tyrosine kinases and that rescue is possible with Csk and Src, we presume that the chemical rescue approach will be generally applicable to the tyrosine kinase family, which contains 90 to 100 members in humans. Because Csk chemical rescue is less efficient than that of Src (*7*), further rescue optimization may be required for specific cases.

This work also suggests that one day it may be possible to find small molecules that rescue disease-related mutant enzymes in people. Notably, the conserved Arg residue investigated here is mutated in at least two genetic diseases involving tyrosine kinases: piebaldism (c-Kit) and agammaglobulinemia (Btk) (*30*, *31*). Screening for small molecules that complement these mutations may ultimately offer hope for the treatment of such diseases.

References and Notes

1. Y. W. Hwang, D. L. Miller, *J. Biol. Chem.* **262**, 13081 (1987).
2. P. J. Belshaw, J. G. Schoepfer, K.-Q. Liu, K. L. Morrison, S. L. Schreiber, *Angew. Chem. Int. Ed. Engl.* **34**, 2129 (1995).
3. M. A. Shogren-Knaak, P. J. Alaimo, K. M. Shokat, *Annu. Rev. Cell Dev. Biol.* **17**, 405 (2001).

4. C. S. Craik, S. Roczniak, C. Largman, W. J. Rutter, *Science* **237**, 909 (1987).
5. P. Carter, J. A. Wells, *Science* **237**, 394 (1987).
6. M. D. Toney, J. F. Kirsch, *Science* **243**, 1485 (1989).
7. D. M. Williams, D. Wang, P. A. Cole, *J. Biol. Chem.* **275**, 38127 (2000).
8. W. E. Lowry *et al.*, *Dev. Cell* **2**, 733 (2002).
9. T. J. Yeatman, *Nat. Rev. Cancer* **4**, 470 (2004).
10. M. C. Frame, V. J. Fincham, N. O. Carragher, J. A. Wyke, *Nat. Rev. Mol. Cell Biol.* **3**, 233 (2002).
11. Materials and methods are available as supporting material on *Science* Online.
12. K. Iguchi, S. Usui, R. Ishida, K. Hirano, *Apoptosis* **7**, 519 (2002).
13. R. A. Klinghoffer, C. Sachsenmaier, J. A. Cooper, P. Soriano, *EMBO J.* **18**, 2459 (1999).
14. J. H. Hanke *et al.*, *J. Biol. Chem.* **271**, 695 (1996).
15. Y. Wang *et al.*, *Nature* **434**, 1040 (2005).
16. F. A. Suprynowicz, A. Baege, I. Sunitha, R. Schlegel, *Oncogene* **21**, 1695 (2002).
17. A. V. Kazansky, J. M. Rosen, *Cell Growth Differ.* **12**, 1 (2001).
18. R. Amanchy, D. E. Kalume, A. Pandey, *Sci. STKE* **267**, pl2 (2005).
19. M. T. Brown, J. A. Cooper, *Biochim. Biophys. Acta* **1287**, 121 (1996).
20. K. Shah, K. M. Shokat, *Chem. Biol.* **9**, 35 (2002).
21. L. Li, D. L. Guris, M. Okura, A. Imamoto, *Mol. Cell. Biol.* **23**, 2883 (2003).
22. S. M. Feller, *Oncogene* **20**, 6348 (2001).
23. R. L. Malek *et al.*, *Oncogene* **21**, 7256 (2002).
24. K. Paz, N. D. Socci, E. van Nimwegen, A. Viale, J. E. Darnell, *Oncogene* **23**, 8455 (2004).
25. D. Fambrough, K. Mcclure, A. Kazlauskas, E. S. Lander, *Cell* **97**, 727 (1999).
26. P. A. Bromann, H. Korkaya, S. A. Courtneidge, *Oncogene* **23**, 7957 (2004).
27. N. H. Tran, J. A. Frost, *J. Biol. Chem.* **278**, 11221 (2003).
28. D. D. Schlaepfer, C. R. Hauck, D. J. Sieg, *Prog. Biophys. Mol. Biol.* **71**, 435 (1999).
29. J. G. Bell, J. A. Wyke, I. A. MacPherson, *J. Gen. Virol.* **27**, 127 (1975).
30. R. A. Spritz, P. Beighton, *Am. J. Med. Genet.* **75**, 101 (1998).
31. M. Vihinen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12803 (1994).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5765/1293/DC1
Materials and Methods
Figs. S1 to S7
References

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Chimpanzees Recruit the Best Collaborators

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Humans collaborate with non-kin in special ways, but the evolutionary foundations of these collaborative skills remain unclear. We presented chimpanzees with collaboration problems in which they had to decide when to recruit a partner and which potential partner to recruit. In an initial study, individuals recruited a collaborator only when solving the problem required collaboration. In a second study, individuals recruited the more effective of two partners on the basis of their experience with each of them on a previous day. Therefore, recognizing when collaboration is necessary and determining who is the best collaborative partner are skills shared by both chimpanzees and humans, so such skills may have been present in their common ancestor before humans evolved their own complex forms of collaboration.

Human society depends on people's ability to collaborate with unrelated individuals in a flexible manner (*1*, *2*). From a young age, human children recognize when they need help in solving a problem, actively recruit collaborators, come to agreements about what type of actions to perform jointly, and recognize others' roles while coordinating their efforts to ensure success (*3*, *4*). Adult humans maintain long-term collaborative partnerships with nonkin by actively monitoring the roles of individuals during collective efforts and basing future collaborations on individual contributions (*5*, *6*).

Although it is clear that human collaborative skills are exceptional, if not unique, in their frequency and complexity, the phylogenetic origins of such skills remain unclear. Of special importance in attempting to identify these origins are humans' nearest primate relatives, such as chimpanzees [see (*7*–*14*) for studies of cooperation in other mammals]. Observations from the wild suggest that chimpanzees possess some collaborative skills; specifically, they may know both when they need a collaborator and something about how they should collaborate. For example, chimpanzees hunt monkeys in groups more often when prey are in dense forest canopy, with many escape routes, than in broken canopy, when escape routes are more limited and individual hunting might be successful (*15*, *16*). During group hunts, chimpanzees seem to coordinate their positions within tree(s) so as to surround monkey prey (*15*, *16*). During risky intergroup encounters, chimpanzees approach the area from which a strange male has called only if their party includes enough adult males to outnumber the rivals (*17*). In addition, chimpanzees may use their social experience to make judgements about the quality of different collaborative partners. Thus, male chimpanzees form long-term alliances with other individuals, jointly defending their territory from other groups and ensuring their access to females within their own group (*15*, *18*). Chimpanzees also tend to reward their favored partners with reciprocal social attention, support, and valuable resources such as meat and mating opportunities. Such preferential treatment of favored partners may maintain long-term collaborative partnerships, because noncollaborators suffer when they are excluded from potential collaborative interactions (*19*–*21*).

However, it is difficult to determine the precise cognitive skills underlying chimpanzees' cooperative activities through natural observations alone. That is, it remains plausible that group hunts may simply consist of the independent yet simultaneous actions of multiple individuals who have little, if any, regard for or understanding of the roles of others in ensuring mutual success (*7*, *16*). Similarly, in most observational studies examining the maintenance of collaborative relations through reciprocal exchange, these interactions can be explained as by-products of symmetrical attraction or aversion between individuals; such symmetry-based reciprocation does not necessarily involve a precise accounting of costs and benefits when choosing to collaborate with different individuals (*22*). Experimental studies of chimpanzee collaboration are also inconclusive, because the few such studies that have been conducted found very modest collaborative skills when individuals were not explicitly trained [(*23*–*25*); see (*11*–*14*) for experimental studies with other primate species]. Moreover, no experiments have ever examined whether chimpanzees can recognize when they need a collaborative partner and whether they can identify, remember, and then preferentially recruit the most effective partner available to them.

In contrast to previous studies, a recent experiment found that most captive chimpanzees can spontaneously (without training) solve a collaborative problem, as long as their social relationship is controlled and they are paired with a tolerant partner (*26*). This new finding raises the possibility of presenting chimpanzees with more complex collaborative situations in order to examine which skills found in humans are derived or inherited. In the current

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study, we investigated whether chimpanzees (i) know when collaboration is necessary and (ii) choose the more effective of two potential collaborators, based on previous experience with each of them.

In the first experiment, eight semi–free-ranging chimpanzees at Ngamba Island Chimpanzee Sanctuary in Uganda were given the opportunity to recruit a collaborative partner when they either (i) needed help in solving a food retrieval problem or (ii) did not need help in solving a food retrieval problem. Subjects were introduced to a "key" (a wooden peg) that could be removed only from inside the testing room (*27*). The key locked a sliding door between the testing room and an adjacent room. If the key was removed by the subject, the sliding door could easily be opened manually (Fig. 1). Subjects were also introduced to a feeding platform in a separate session (*27*). The feeding platform was placed next to the testing room but out of the reach of the subject(s), and both feeding dishes were always baited with equal amounts of food. A rope was then threaded through two metal loops anchored to the feeding platform, and both ends of the rope were extended into the testing room. Therefore, in each trial if the subject(s) pulled both ends of the rope simultaneously, the feeding platform could be pulled within reach; however, if only one end of the rope was pulled, the rope became unthreaded and the food was lost (Fig. 1) (*27*, *28*).

After separate introductions to the key and pulling task (*27*), subjects participated in two types of test conditions. In the collaboration condition, the subject and partner watched from separate rooms adjacent to the testing room as the baited food platform and ropes were positioned so that the two ends of the rope were placed 3 m apart: too far for one individual to pull both simultaneously. The subject was then released into the testing room while the partner remained "locked" in the adjacent room, unless the subject chose to release her by removing the key and unlocking the door so that they could work together to obtain the food. The solo condition differed from the collaboration condition only in that the two ends of the rope were placed 55 cm apart, so that a subject did not need help in obtaining the food because she could potentially pull both rope ends simultaneously by herself [movie S1 (*27*)]. Subjects were tested in two test sessions in which they received a maximum of 12 trials per condition during each session.

Overall, when both sessions are considered together, subjects unlocked the door to recruit their partner significantly more often in the collaboration condition, when they needed assistance to obtain the food, than in the solo condition, when they did not (Table 1; $t = 7.27$, df = 7, $P < 0.001$, paired t test). As individuals, seven of eight subjects recruited significantly more in the collaboration than the solo condition across the two sessions (Table 1; Fisher's exact test, $P < 0.05$). A 2 × 2 repeated-measures analysis of variance (ANOVA) (condition × session) revealed that the six subjects opened the door significantly more in the collaboration condition [$F(1,5) = 71.42$, $P < 0.001$], whereas there was no effect of session and a significant interaction between condition and session [$F(1,5) = 8.81$, $P < 0.031$], with subjects' recruitment in the collaboration condition increasing in the second session ($t = 2.39$, df = 5, $P < 0.031$, paired sample t test).

Subjects' preference for recruiting a collaborator when needed appeared relatively spontaneously within the first session, as each subject began recruiting a partner. In the session in which they started to open the door, five of eight subjects, as individuals, opened it significantly more in the collaboration condition (Table 1). A repeated-measures ANOVA also found that there was little change in subjects' preference for recruiting in the collaboration condition during the second session (the session in which all subjects recruited), because there was only a main effect of condition [$F(1,7) = 56$, $P < 0.001$] but no effect of trial (comparing the first three trials of a given condition to the last three trials of that condition) or interaction between trial and condition.

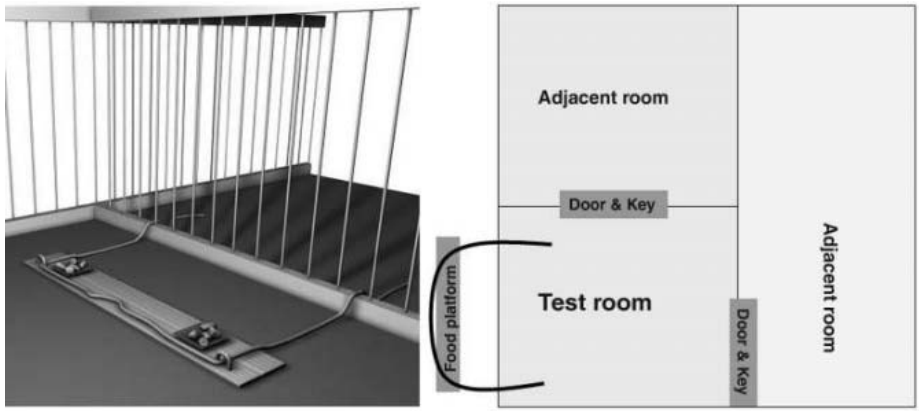

Fig. 1. Experimental setup. The baited food platform, metal loops, threaded rope extended into the test room, room layout used in the two studies, and placement of the food platform. In experiment 1, the subject was released from an adjacent room into the testing room, while the partner was "locked" in another adjacent room that only the subject could open with a key (a wooden peg) from inside the testing room. In experiment 2, the subject was released directly into the test room from a third adjacent room not represented here, while two potential partners were each locked in one of two adjacent rooms that the subject could again open with a key.

Table 1. Percentage of trials in session 1, session 2, and both sessions combined in which subjects opened the door for the potential partner in the adjacent room in experiment 1 (*$P < 0.05$, **$P < 0.01$, ***$P \leq 0.001$, Fisher's exact test). Scores are in bold if during the first session in which a subject began recruiting the subject also had an immediate preference for recruiting in the collaboration condition. In the first session, two of the four subjects who recruited partners did so significantly more often (as individuals) in the collaboration condition. In the second session, four subjects recruited partners for the first time and three of them did so significantly more in the collaboration condition. Of these three subjects, one did not participate in session 1, whereas the other two never recruited in session 1 but discovered the method in a brief warm-up between sessions (*27*). Moreover, four of these five subjects, in the first testing block (the three-trials-block) in which they started recruiting, did so in the collaboration condition one to three times and never in the first three trials of the solo condition. (Note: if subjects did not discover the possibility of opening the door in session 1, they were given only six trials in each condition of session 1.)

Subject name	Session 1		Session 2		Combined	
	Collaborate	Solo	Collaborate	Solo	Collaborate	Solo
Namukisa	0	0	**100*** **	0	66.7***	0
Kalema	0	0	**100*** **	8.3	66.7***	4.6
Okech	**91.7*** **	8.3	100***	33.3	95.8***	20.8
Baluku	**58.3** **	0	100***	25	79.2***	12.5
Umugenzi	25	16.7	100***	16.7	62.5***	16.7
Indi	100	100	75***	8.3	83.3**	38.8
Bili	—	—	**100***	66.7	100*	66.7
Asega	—	—	100	83.3	100	83.3
Combined	45.8	20.8	96.8	30.2	73.4	30.4

Given the finding that chimpanzees recruit a collaborator only when needed, a second experiment was designed to test whether they can also learn to recruit the more effective of two partners on the basis of a limited number of interactions with each of them. Six chimpanzees, who had previously participated in experiment 1, were first reintroduced to the key mechanism and shown that now both sliding doors connecting to two rooms adjacent to the testing room could be opened by use of the key in each door (Fig. 1) (*27*). Then subjects were all introduced and tested with the same two potential collaborators. These two potential collaborators had previously demonstrated very different levels of success in pulling the food tray with others (*27*) and on this basis were designated as the more effective and the less effective partner. Subjects in experiment 2 had not previously collaborated with either of the two potential partners in this context [see (*27*)]. The testing procedure was identical to that in the collaborative condition from experiment 1, with the exception that in each trial, the more and less effective collaborators were in the two separate rooms adjacent to the testing room and both doors were locked with a key, so that the subject could open either door from inside the testing room (Fig. 1).

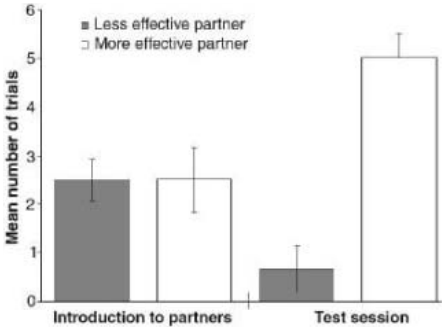

Fig. 2. The mean number of times (±SEM) that the subjects chose the less or more effective partner in the introductory and test sessions of experiment 2.

Table 2. The percentage of trials in which subjects responded to a previous success by staying with the same partner (win-stay) and to a failure by shifting partners (lose-shift) versus the percentage of trials in which subjects responded by shifting partners after a success (win-shift) and staying after a failure (lose-stay). Several subjects, as individuals, made win-stay and lose-shift responses significantly more than win-shift and lose-stay responses (Fisher's exact test, $*P < 0.05$).

Subject	Win-stay and lose-shift	Win-shift and lose-stay
Okech	90.9*	9.1
Bili	81.81*	18.19
Umugenzi	80*	20
Asega	83.33	16.67
Namukisa	55.56	44.44
Baluku	54.54	45.46
Overall	74.34	25.64

Subjects participated in two sessions. In an introductory session, subjects were introduced to the potential collaborators by being released into the testing room and allowed to recruit either the more or less effective collaborator (by choosing whose door to open) in six consecutive trials. In the test session, occurring on a subsequent day, subjects were again released into the testing room during six trials and allowed to chose which of the two partners to recruit for the job of pulling in the food platform [movie S1 (*27*)].

We conducted a 2 × 2 repeated-measures ANOVA (partner × session) to determine subjects' recruitment preferences and whether these preferences changed from the introduction to the test session. Overall, subjects preferred to recruit the more effective partner over the less effective partner [$F(1,5) = 13$, $P = 0.015$]. But this preference must be interpreted in the context of a significant interaction between partner and session [$F(1,5) = 9$, $P = 0.027$]: subjects' preference for the more effective partner in the test session only. Indeed, as Fig. 2 shows, subjects had no preference in the introductory session, whereas in the test session they chose the more effective partner over the less effective partner almost exclusively (in 30 out of 34 trials in which a partner was recruited). Subjects' choices on the first trial of each of their two sessions corroborate this change in preference: Five of six subjects first recruited the less effective partner in the introduction session, whereas five of six subjects first recruited the more effective collaborator in the test session [$P = 0.039$, exact bivariate binomial test, two-tailed (*27*)]. The relative difference in the effectiveness of the two potential partners who were designated more and less effective on the basis of a pretest (*27*) was again observed in the experiment: Subjects were significantly more successful at retrieving the food with the more effective partner than with the less effective partner ($t = 4.36$, df = 5, $P < 0.004$, paired t test).

The change in subjects' preferences between the less and more effective partners across testing sessions suggests the possibility that subjects were tracking their relative level of success with each of the partners and subsequently based their recruitment decisions on previous outcomes. Support for this interpretation comes from the finding that the number of times that a given subject recruited the more effective partner relative to the less effective partner correlates with the level of success that the subject had in retrieving the food with the more effective partner relative to the less effective partner ($r_s = 0.838$, $n = 6$, $P = 0.019$, Spearman's rho). This means that subjects who had the highest level of success with the more effective partner also chose him relatively more. In addition, a trial-by-trial analysis reveals that subjects were basing recruitment choices in a given trial on the outcome of the preceding trial. For this analysis, each subject's choice on each trial (after the first) was classified as either staying with the choice of partner from the previous trial or shifting to the other partner. Overall, subjects responded to the outcome of the previous trial by staying after success and shifting after failure significantly more than they responded by staying after failure and shifting after success ($t = 3.87$, df = 5, $P = 0.006$; Table 2). In addition, a 2 × 2 repeated-measures ANOVA (previous trial: success or fail × next choice: stay or shift) revealed that when subjects succeeded in a trial, they stayed with the same partner on the next trial more often than they shifted, whereas when they failed in a trial, they more often shifted than stayed [a significant interaction between the previous trial and the next choice: both sessions, $F(1,5) = 12.25$, $P = 0.017$; introductory session, $F(1,5) = 15.16$, $P = 0.011$; Fig. 3]. The correlation and trial-by-trial analysis suggest that subjects' change in partner preference was caused by subjects basing future recruitment choices on the outcome of previous collaboration attempts with each partner.

Finally, it is important to note that failures to obtain the food (with both partners) in the

Fig. 3. The mean percentage of trials (±SEM) in both sessions of experiment 2 in which subjects responded to a failure (lose) or success (win) on a previous trial by staying with the same partner again or shifting to the other partner on the next trial. In addition to the significant interaction found between previous outcomes and partner choice on the next trial, pairwise comparisons between choices after successful trials reveal that subjects stayed with the same partner after success more than they shifted (both sessions: $t = 1.972$, df = 5, $P = 0.006$; intro session: $t = 1.67$, df = 5, $P = 0.08$, paired t test). Likewise, comparisons between choices after failed trials reveal that subjects shifted to the other partner after a failure more than they stayed (both sessions: $t = 1.97$, df = 5, $P = 0.053$; intro session: $t = 2.93$, df = 5, $P = 0.017$, paired t test).

introductory session were due not to the subject's actions but to the partner, because he either pulled the rope prematurely or did not enter the test room (with the less effective partner causing significantly more errors: $t = 2.076$, df = 5, $P = 0.047$, paired t test). This raises the additional possibility that subjects may have attributed collaboration failures to the behavior of the partner, and in part developed their preference for the more effective partner because he caused the least errors.

The current results demonstrate that chimpanzees understand when it is necessary to recruit a collaborator and can identify and choose the better of two potential collaborators after only a small number of interactions with each. In the first study, chimpanzees almost always unlocked the door for a potential partner when they needed help in retrieving a food tray, whereas these same individuals almost never unlocked the partner's door when they could retrieve the food on their own. Even though these subjects never had the opportunity to open a door for another individual in a collaborative situation before the test, the majority of them did so on their very first trial when collaboration was necessary. This indicates that chimpanzees can quickly adapt a recently learned skill (removing the key) for a novel purpose (initiating a collaborative activity). In the second experiment, chimpanzees used a win-stay/lose-shift strategy while interacting with two partners that differed in their collaborative skills. Although subjects had not collaborated with either potential partner previously, they learned to choose the more effective partner as a collaborator: In an initial introductory session, subjects did not prefer the more effective partner, whereas in a subsequent session, subjects almost exclusively chose to recruit the more effective partner (showing this change of preference in their very first trial of the test). Therefore, subjects may have remembered the two partners' collaborative performance in the introduction and then developed their preference for the more effective partner because of their higher success rate with him [as opposed to making choices based on other behaviors or on intrinsic differences between the potential partners (*29*, *30*)]. It is even possible that subjects developed their preferences for the more effective partner after attributing more failures to the less effective partner in the introduction. Regardless, subjects' ability to quickly develop and remember a preference for the most effective partner resulted in fewer opportunities for the less effective partner to collaborate, as well as higher rates of food intake for subjects. This mechanism probably facilitates the maintenance of some cooperative strategies, such as reciprocal relationships between dyads.

Overall, the current findings, in conjunction with previous natural observations, challenge the hypothesis that cooperative behaviors in chimpanzees do not represent active collaboration in which individuals intentionally choose with whom and when to work together (*7*, *20*). The implication is that human forms of collaboration are built on a foundation of evolutionary precursors that are present in chimpanzees and a variety of other primate species (*10*–*14*). Further study of chimpanzee, and perhaps bonobo, collaboration is necessary to more precisely identify the derived forms of human collaboration that have arisen since our species split from our last common ancestor with nonhuman apes (*3*, *6*).

References and Notes

1. E. Wilson, *Sociobiology: The New Synthesis* (Harvard Univ. Press, Cambridge, MA, 1975).
2. J. Henrich *et al.*, *Foundations of Human Sociality* (Oxford Univ. Press, Oxford, 2004).
3. M. Tomasello, M. Carpenter, J. Call, T. Behne, H. Moll, *Behav. Brain Sci.,* in press.
4. F. Warneken, F. Chen, M. Tomasello, *Child Dev.,* in press.
5. R. Trivers, *Q. Rev. Biol.* **46**, 35 (1971).
6. E. Fehr, U. Fischbacher, *Trends Cogn. Sci.* **8**, 185 (2004).
7. M. Tomasello, J. Call, *Primate Cognition* (Oxford Univ. Press, Oxford, 1997).
8. A. Harcourt, F. de Waal, *Coallitions and Alliances in Humans and Nonhuman Animals* (Oxford Univ. Press, Oxford, 1992).
9. A. Engh, E. R. Siebert, D. Greenberg, K. Holekamp, *Anim. Behav.* **69**, 209 (2005).
10. J. Silk, *Anim. Behav.* **58**, 45 (1999).
11. E. Stammbach, *Behaviour* **107**, 241 (1988).
12. F. B. M. de Waal, M. Berger, *Nature* **404**, 563 (2000).
13. M. Hauser, M. K. Chen, F. Chen, E. Chuang, *Proc. R. Soc. London Ser. B* **270**, 2363 (2003).
14. Y. Hattori, H. Kuroshima, K. Fujita, *J. Comp. Psychol.* **119**, 335 (2005).
15. C. Boesch, H. Boesch-Achermann, *The Chimpanzees of the Tai Forest* (Oxford Univ. Press, Oxford, 2000).
16. D. Watts, J. Mitani, in *The Diversity of Chimpanzees*, C. Boesch, G. Hohmann, L. Marchant, Eds. (Cambridge Univ. Press, Cambridge, 2002), pp. 244–255.
17. M. L. Wilson, M. D. Hauser, R. W. Wrangham, *Anim. Behav.* **61**, 1203 (2001).
18. R. W. Wrangham, *Yearb. Phys. Anthropol.* **42**, 1 (1999).
19. F. B. M. de Waal, L. M. Luttrell, *Ethol. Sociobiol.* **9**, 101 (1988).
20. C. Hemelrijk, A. Ek, *Anim. Behav.* **41**, 923 (1991).
21. N. F. Koyama, C. Caws, F. Aureli, *Int. J. Primatol.,* in press.
22. J. Stevens, M. Hauser, *Trends Cogn. Sci.* **8**, 60 (2004).
23. D. J. Povinelli, D. K. O'Neill, in *Understanding Other Minds: Perspectives from Developmental Cognitive Neuroscience*, S. Baron-Cohen, H. Tager-Flusberg, D. J. Cohen, Eds. (Oxford Univ. Press, Oxford, 2000), pp. 459–487.
24. M. P. Crawford, *Comp. Psychol. Monogr.* **14**, 1 (1937).
25. R. Chalmeau, *Primates* **35**, 385 (1994).
26. A. P. Melis, B. Hare, M. Tomasello, *Anim. Behav.,* in press.
27. For further details of the methodology, please see the supporting material on *Science* Online.
28. S. Hirata, *Hattatsu* **95**, 103 (2003).
29. Before each subject's choice in experiment 2, the behavior of the two potential partners was coded in order to examine whether subjects might have preferentially chosen the more effective partner more in the second session solely on the basis of a change in behavior in one or both partners while waiting for a choice to be made (*27*). A 2 × 2 repeated-measures ANOVA (session × partner) revealed no effect of session and no interaction between partner and session, but a main effect of the different partners' tendency to sit directly in front of the door during the 5 s before the subject's choice [$F(1,5) = 20.59$, $P < 0.006$]. In addition, the same analysis again revealed no effect of session and no interaction between partner and session, but a main effect of the different partners' tendency to shake the door before a subject's choice [$F(1,5) = 9.31$, $P < 0.028$]. Therefore, although the more effective partner was more often directly in front of the door and shaking it before being chosen, the behavior of the two potential partners did not change across the two sessions. This makes it difficult to explain the subject's change in preferences between sessions as being based on a change in the behavior of the potential partners in the different sessions. In addition, in the introduction session, equal numbers of subjects were successful at working with both of the potential partners in at least one trial. Four of six subjects succeeded once with the less effective partner and four of six subjects succeeded at least once with the more effective partner (in one case, the more effective partner was never chosen by the subject). This suggests that subjects' social relations were friendly enough in all potential dyads to allow for cooperation with either potential partner (that is, subjects were not simply too afraid of the less effective partner to choose him).
30. The two potential partners differed from one another in a number of intrinsic characteristics (such as size, age, dominance, etc.), and so it is important to establish that the relative effectiveness of the partner was indeed a critical variable in the subjects' choice of partner and change of preferences between sessions. First, the intrinsic characteristics of group mates with which subjects should be familiar probably did not change between sessions. Therefore, it seems more likely that subjects' change of preference occurred as they learned about the two partners' relative effectiveness as collaborators, because they had not previously interacted with them in this context before the introductory session. Second, it also seems that the partners' behavior that might indicate eagerness to participate, as measured observationally, also did not change across sessions (*29*). Third, subjects would not be predicted to base their choices of partners on the outcomes of previous trials if their choices were overwhelmingly influenced by intrinsic differences between potential partners, yet subjects appear to have used a win-stay/lose-shift strategy throughout both sessions. It seems that subjects applied this same strategy with both partners. Subjects also responded to previous outcomes by staying after success and shifting after failure significantly more than staying after failure and shifting after success with both of their potential partners (less effective partner: $t = 3.8$, df = 5, $P < 0.007$; more effective partner: $t = 2.0$, df = 5, $P = 0.051$, paired sample t test; fig. S2), with no difference between how often this strategy was used with either subject ($t = 0.525$, df = 5, P = NS, paired t test).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5765/1297/DC1
Materials and Methods
Figs. S1 and S2
Table S1
References
Movie S1

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Altruistic Helping in Human Infants and Young Chimpanzees

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Human beings routinely help others to achieve their goals, even when the helper receives no immediate benefit and the person helped is a stranger. Such altruistic behaviors (toward non-kin) are extremely rare evolutionarily, with some theorists even proposing that they are uniquely human. Here we show that human children as young as 18 months of age (prelinguistic or just-linguistic) quite readily help others to achieve their goals in a variety of different situations. This requires both an understanding of others' goals and an altruistic motivation to help. In addition, we demonstrate similar though less robust skills and motivations in three young chimpanzees.

Helping is an extremely interesting phenomenon both cognitively and motivationally. Cognitively, to help someone solve a problem, one must know something about the goal the other is attempting to achieve as well as the current obstacles to that goal. Motivationally, exerting effort to help another person—with no immediate benefit to oneself—is costly, and such altruism (toward non-kin) is extremely rare evolutionarily. Indeed, some researchers have claimed that humans are altruistic in ways that even our closest primate relatives are not. A powerful method to test this idea is to directly compare human infants and our closest primate relatives (chimpanzees) on their propensity to help. Such a comparison may enable us to distinguish aspects of altruism that were already present in the common ancestor of chimpanzees and humans from aspects of altruism that have evolved only in the human lineage. To date, no experimental studies have systematically tested human infants and chimpanzees in a similar set of helping situations.

A number of studies have demonstrated that young children show concern (empathy) for others in distress. Preschool-age children and even infants (1 to 2 years of age) occasionally attempt to respond to the emotional needs of others, for example, by comforting someone who is crying (*1*–*10*). In contrast, there are no experimental studies with infants that have systematically investigated instrumental helping—providing help to people who are faced with an instrumental problem and are unable to reach their goal (*11*–*13*).

In the current study we presented 24 18-month-old infants with 10 different situations in which an adult (a male experimenter) was having trouble achieving a goal. This variety of tasks presented the children with a variety of difficulties in discerning the adult's goal and his problems in reaching the goal. These situations fell into four categories: out-of-reach objects, access thwarted by a physical obstacle, achieving a wrong (correctable) result, and using a wrong (correctable) means (Table 1) (movies S1 to S4). For each task, there was a corresponding control task in which the same basic situation was present but with no indication that this was a problem for the adult (*14*). This ensured that the infant's motivation was not just to reinstate the original situation or to have the adult repeat the action, but rather to actually help the adult with his problem. After the occurrence of the problem in each task (e.g., marker drops on floor), there were three phases: The experimenter focused on the object only (1 to 10 s), then alternated gaze between object and child (11 to 20 s), and in addition verbalized his problem while continuing to alternate gaze (e.g., "My marker!"; 21 to 30 s). In control trials, he looked at the object with a neutral facial expression for 20 s. In no case did the infant receive any benefit (reward or praise) for helping. Each individual was tested in all 10 tasks, a subsample of 5 tasks administered as experimental and 5 as control conditions (in systematically varied order). Thus, in each task 12 children were tested in the experimental condition and 12 others in the control condition for a between-subjects comparison.

Results showed that infants helped the adult (the infant performed the target behavior significantly more in experimental than in control conditions) in 6 of the 10 tasks—at least one for each category (Fig. 1). They handed him several out-of-reach objects (but not if he had discarded them deliberately); they completed his stacking of books after his failed attempt (but not if his placement of the books appeared to meet his goal); they opened the door of a cabinet for him when his hands were full (but not if he struggled toward the top of the cabinet); and they retrieved an inaccessible object for him by opening a box using a means he was unaware of (but not if he had thrown the object inside the box on purpose). Analyzed by individual, 22 of the 24 infants helped in at least one of the tasks. It is noteworthy that they did so in almost all cases immediately (average latency = 5.2 s), before the adult either looked to them or verbalized his problem (84% of helping acts within the initial 10-s phase). Thus, the experimenter never verbally asked for help, and for the vast majority of helping acts, eye contact (as a subtle means of soliciting help) was also unnecessary.

Experimental studies on altruistic behaviors in nonhuman primates are scarce. There are anecdotal reports of possible instances of helping (*15*–*17*) and some experiments demonstrating empathic intervention by various monkey species when another individual is displaying emotional distress (but no experiments with apes) (*18*). However, there are no studies, to our knowledge, of nonhuman primates helping others who are struggling to achieve their goals (instrumental helping) (*19*, *20*). In two recent experiments, chimpanzees were given the opportunity to deliver food to a conspecific (*21*, *22*), but again that conspecific was not trying to solve a problem in which the subject could help instrumentally [see also (*23*)]. Results were negative. But it is possible that altruism would be more likely when it involves objects other than food, because chimpanzees often compete over food and the drive to acquire food for themselves might preclude their capacity to act on behalf of others. In the current study, therefore, we gave the same basic tasks of instrumental helping given to the infants, with some minor modifications, to three young chimpanzees (*Pan troglodytes*, one of humans' two closest living relatives). These individuals were 36, 54, and

Table 1. Examples of problems used in child study.

Category	Task	Problem
Out-of-reach	Marker	The adult accidentally drops a marker on the floor and unsuccessfully reaches for it (experimental) or intentionally throws a marker on the floor (control).
Physical obstacle	Cabinet	The adult wants to put magazines into a cabinet, but the doors are closed so that he bumps into it (experimental) versus bumping into the doors as he tries to lift the magazines onto the cabinet (control).
Wrong result	Book	A book slips from a stack as the adult attempts to place it on top of the stack (experimental) or he places it next to the stack (control).
Wrong means	Flap	A spoon drops through a hole and the adult unsuccessfully tries to grasp it through the small hole, ignorant of a flap on the side of the box (experimental). Alternatively, he throws the spoon in the box on purpose (control).

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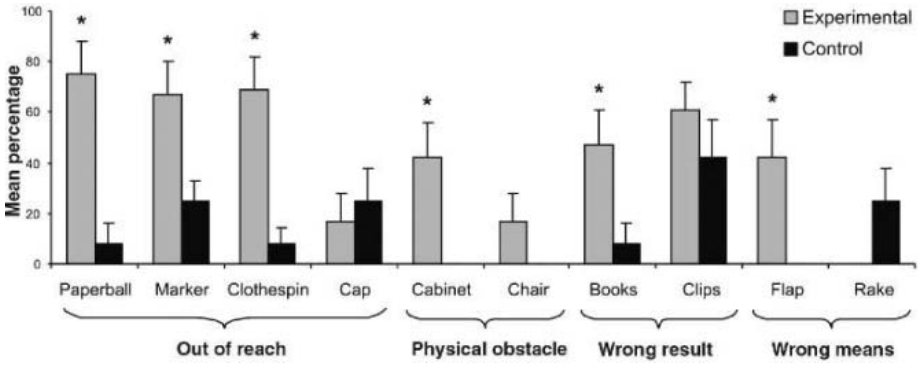

Fig. 1. Mean percentage of target behaviors as a function of task and condition. In tasks with multiple trials, the mean percentage of trials with target behavior per total number of trials was computed for each individual. Independent-sample *t* tests (df = 22) revealed significant differences between conditions for the tasks Paperball ($t = 4.30$, $P < 0.001$), Marker ($t = 2.70$, $P < 0.05$), Clothespin ($t = 4.38$, $P < 0.001$), Books ($t = 2.33$, $P < 0.05$), and Cabinet ($t = 3.08$, $P < 0.01$). For the Flap task with only one trial per individual, we computed Fisher's exact test ($N = 24$, $P < 0.05$). In these six tasks, children performed the target behavior significantly more often in the experimental than in the control condition. No difference between conditions was found for the tasks Clips ($t = 1.04$, $P = 0.31$), Cap, Chair, and Tool, Fisher's exact tests ($N = 24$), $P = 1.0$, 0.48, and 0.22, respectively. Error bars represent SE; $^*P < 0.05$.

54 months of age at the time of testing and had been raised their whole life by humans. Each chimpanzee performed both conditions of each task in two different sessions on consecutive days. They were tested by a highly familiar human caretaker with whom they spent time on a daily basis.

The chimpanzees helped in some of the tasks (movies S5 to S8). All three chimpanzees helped reliably in the five tasks involving reaching: Across all such trials, the chimpanzees could retrieve objects for the human from 0 to 13 times in both the experimental and control conditions. The scores of the three individuals (experimental, control) were as follows: Alex, 5, 0; Alexandra, 10, 3; Annet, 9, 0 (each pair is significantly different from a chance distribution: Fisher's exact test, $P = 0.039$; $P = 0.017$, $P = 0.0005$, respectively). Because it was more difficult to control the behavior of the chimpanzees than that of the children, the human had to call each one by name to pay attention more often and sooner in the process. Nonetheless, when the chimpanzees helped, they did so relatively quickly (average latency = 12.9 s of reaching for the object), with each of the three individuals helping the human from 4 to 7 times across all tasks before she verbalized anything. As with the human infants, they did so without receiving any benefit (reward or praise) for helping (although they retained the object in their possession for some seconds before handing it over more often than did the children).

However, the chimpanzees did not help the human reliably in the other types of tasks—that is, in those involving physical obstacles, wrong results, or wrong means. In a follow-up study, we gave them two additional tasks of these types—designed to make the human's problem especially salient and with more time for a response—and they still did not help in these tasks (*14*). Presumably, when someone is reaching with an outstretched arm toward an object, the goal is in principle easier to understand and the kind of intervention follows straightforwardly. This could explain why out-of-reach tasks (in contrast to the other scenarios) elicited more helping by children and the only instances of helping by chimpanzees. Children and chimpanzees are both willing to help, but they appear to differ in their ability to interpret the other's need for help in different situations.

These experimental results demonstrate instrumental helping (toward goals) in a nonhuman primate. It is possible that helping behaviors are more likely when they involve objects that are not food, and that this explains why we obtained positive results when others, using different tasks involving food, have found negative results. It should also be noted that the chimpanzees of the current study, unlike those in (*21*, *22*), were helping not a conspecific but a human. This might be important because chimpanzees are extremely competitive with one another (*24*, *25*), but when they grow up interacting with humans, they seem to develop some more cooperative skills and motivations as well. Although our chimpanzees had been rewarded in the past for handing humans objects already in their possession upon request, they had not been encouraged to retrieve, nor rewarded for retrieving, out-of-reach objects for humans.

The human infants helped much more, and they did so for an adult they had just met (who was clearly not kin). Of special note, they helped in four different kinds of situations, whereas the chimpanzees helped in only one. This could be due to a greater propensity to help in children, or to children's more sophisticated cognitive skills in discerning the goal of the other in a variety of different situations. Infants 18 months of age are too young to have received much verbal encouragement for helping from parents. However, even if they had received some prior encouragement, many of the current tasks would have been unfamiliar for them, and the recipient of the help was an unfamiliar adult as well. In any case, viewed from a larger evolutionary perspective, the facts that human parents encourage their children to help others and that children comply by helping (even before they are linguistic) are noteworthy as the teaching and learning of prosocial norms.

A number of theorists have claimed that human beings cooperate with one another and help one another (especially non-kin) in ways not found in other animal species (*26*–*28*). This is almost certainly so, and the current results demonstrate that even very young children have a natural tendency to help other persons solve their problems, even when the other is a stranger and they receive no benefit at all. However, our nearest primate relatives show some skills and motivations in this direction as well, and this suggests that the common ancestor to chimpanzees and humans already possessed some tendency to help before humans began down their unique path of hypercooperativeness (*25*, *29*).

References and Notes

1. D. Bischof-Köhler, in *Infant Development: Perspectives from German-Speaking Countries*, M. E. Lamb, H. Keller, Eds. (Erlbaum, Hillsdale, NJ, 1991), pp. 245–273.
2. D. Bischof-Köhler, *Z. Psychol. Z. Angew. Psychol.* **202**, 349 (1994).
3. D. Bischof-Köhler, *Psychol. Erzieh. Unterr.* **47**, 142 (2000).
4. J. Dunn, P. Munn, *Int. J. Behav. Dev.* **9**, 265 (1986).
5. N. Eisenberg, R. A. Fabes, in *Handbook of Child Psychology: Vol. 3. Social, Emotional, and Personality Development*, W. Damon, N. Eisenberg, Eds. (Wiley, New York, ed. 5, 1998), pp. 701–778.
6. J. E. Grusec, M. Davidov, L. Lundell, in *Blackwell Handbook of Childhood Social Development*, P. K. Smith, C. H. Hart, Eds. (Blackwell, Malden, MA, 2002), pp. 457–474.
7. M. L. Hoffman, *Empathy and Moral Development: Implications for Caring and Justice* (Cambridge Univ. Press, New York, 2000).
8. C. Zahn-Waxler, M. Radke-Yarrow, R. A. King, *Child Dev.* **50**, 319 (1979).
9. C. Zahn-Waxler, M. Radke-Yarrow, E. Wagner, M. Chapman, *Dev. Psychol.* **28**, 126 (1992).
10. D. B. Johnson, *Merrill Palmer Q.* **28**, 379 (1982).
11. Infants and young children readily participate in typical household chores such as cleaning up (*12*) and also sometimes provide information for others (*4*, *13*), but in the studies done to date, there have been no control or baseline conditions to determine whether the children are actually helping others with their goals or just engaging in the activity for its own sake, independently of the other actually needing help.
12. H. L. Rheingold, *Child Dev.* **53**, 114 (1982).
13. U. Liszkowski, M. Carpenter, T. Striano, M. Tomasello, *J. Cogn. Dev.*, in press.
14. See supporting material on *Science* Online.
15. S. M. O'Connell, *Primates* **36**, 397 (1995).
16. F. de Waal, *Good Natured* (Harvard Univ. Press, Cambridge, MA, 1996).

17. B. B. Beck, *Science* **182**, 594 (1973).
18. S. Preston, F. de Waal, *Behav. Brain Sci.* **25**, 1 (2002).
19. In one study, tamarin monkeys sometimes pulled food to within reach of conspecifics, but the other was doing nothing special at that time; that is, it was never attempting to solve a problem (*20*).
20. M. Hauser, M. Chen, F. Chen, E. Chuang, *Proc. R. Soc. London Ser. B* **270**, 2363 (2003).
21. K. Jensen, B. Hare, J. Call, M. Tomasello, *Proc. R. Soc. London Ser. B*, published online 17 January 2006 (10.1098/rspb.2005.3417).
22. J. Silk *et al.*, *Nature* **437**, 1357 (2005).
23. D. L. Wolfe, H. M. Wolfe, *J. Genet. Psychol.* **55**, 137 (1939).
24. B. A. Hare, M. Tomasello, *Anim. Behav.* **68**, 571 (2004).
25. M. Tomasello, M. Carpenter, J. Call, T. Behne, H. Moll, *Behav. Brain Sci.* **28**, 5 (2005).
26. R. D. Alexander, *The Biology of Moral Systems* (de Gruyter, Hawthorne, NY, 1987).
27. E. Fehr, U. Fischbacher, *Nature* **425**, 785 (2003).
28. J. Stevens, M. Hauser, *Trends Cogn. Sci.* **8**, 60 (2004).
29. P. J. Richerson, R. Boyd, *Not by Genes Alone* (Univ. of Chicago Press, Chicago, 2005).
30. We thank the children and their parents for their help; E. Rossi for her assistance with the children; S. Mauritz, C. Richter, and J. Collard as well as the keepers at the Leipzig Zoo for their assistance with the chimpanzees; D. Stahl for statistical advice; and H. Rakoczy and two anonymous reviewers for thoughtful comments on an earlier draft.

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EDITORIAL FEATURE

Working the Systems

Systems biology seeks to connect the dots between molecular data. It's a hot career field, but success requires making connections between disciplines

SEATTLE, WASHINGTON—The Institute for Systems Biology (ISB) here occupies a new building with large, rectangular panels of glass offset by brick. From the outside, the building has a vaguely modular feel, intended, perhaps, to evoke a system of linked components. Glass dominates the façade, providing those inside panoramic views of Seattle's Lake Union and the northwest's turbulent weather. Within, pastel walls and numerous cubbyholes and conference rooms encourage easy communication, and the design itself reflects some social engineering: "To get to the centrifuge, you may have to walk past the computational people," says Alan Aderem, a co-founder of the institute and one of its current directors. When you walk past the computational people, Aderem hopes you'll stop to strike up a conversation.

Six years after the building's construction, ISB remains the ambitious experiment in interdisciplinary research that began when founder and president Leroy Hood concluded that he couldn't arrange a successful marriage between computing and biology in the academic environment of the University of Washington. Six years on, there is no clear definition of systems biology, although most would agree it has something to do with understanding dynamic, molecular-level relationships among biological molecules in living systems. Like the building in Seattle, the new field brings together physiologists, molecular biologists, biochemists, computer scientists, mathematicians, engineers, physicists, and a few other specialists and encourages them to work together to look beyond individual genes and proteins to a holistic view of whole systems—like the view that dominated biology before the advent of molecular biology. But systems biology adds insights and an arsenal of techniques developed over the past half-century. There is little doubt about the power and potential of the systems approach, but have ISB's attempts at architectural, social, and scientific-systems engineering produced a smoothly operating new scientific discipline?

Not yet. "I underestimated how resistant people are to leaving their comfort zones," says John Aitchison, who joined ISB in 2000 from the University of Alberta, Canada. And that, in a nutshell, may define the opportunities and challenges of systems biology, as a field of science and as a career.

Opportunities

No question, systems biology is in heavy demand. "Systems biology is very fashionable. Until it is fully established in all of the major universities, there will be a lot of hires, either new hires or professors that are reminting themselves," predicts David Galas, a researcher at ISB and vice president and chief scientific officer of the Battelle Memorial Institute in Columbus, Ohio. Roger Brent, president and research director of the independent Molecular Sciences Institute (MSI) in Berkeley, California, agrees. "I'd say that this is a time in which a talented young person who demonstrates an ability to make real contributions can pretty close to write their own ticket in terms of what they can do academically and intellectually," he says.

One reason the outlook is so rosy is that "there are really very few" people who have the combination of biological and computational skills to fill those types of positions, says John Barnett, director of the center for immunopathology and microbial pathogenesis at West Virginia University in Morgantown. According to Barnett, those who have joint training "have a really good job market."

Industry has been cautious in embracing the new field, "and rightly so," says Galas. "They want to see how long it will take to benefit them. It's happening, but at a slower rate." The number of industry jobs may increase when the biotech market changes. "At the moment, funding in biotechnology is skewed toward later stages of research, where potential products are in clinical trials. I think where you will see it first is the larger companies that are trying to make long-term plays."

ISB is not a likely long-term career destination, because the institute hires few senior scientists. But it is an excellent training ground, with 50 or so postdoc positions opening every year. And according to Hood, "most people who have come through here have had no problem getting jobs."

In the blood. At the Institute for Systems Biology (*top pictures*), former bioengineer Nathan Price (*above*) looks for early fingerprints of cancer in patterns of blood proteins.

Career Profiles | Resources for Support

Challenges

Despite its opportunities, systems biology can be a difficult field to work in. It relies on collaborations, and ISB and similar centers have struggled to build them. "The differences [between biologists and

CREDITS (TOP): TEAM PHOTOGENIC; (BOTTOM) ISB

A work in progress. Roger Brent says universities are still struggling to embrace the interdisciplinary research that is a hallmark of systems biology.

computational scientists] are really remarkable. They speak and think differently," says Aitchison. "Biologists think of themselves as wise, sagely knowledge banks, and they see computer people as keyboard jockeys. The computer guys think of themselves as mathematics-driven scientists. They think of biologists as lab technicians. [The problem is] getting people to bring appreciation for each other's work to the table. There is the potential for resentment."

Some of the problems have been a surprise. Says ISB's Aderem: "I expected hard-core mathematicians and physicists to have a relatively easy job learning biology because we're all inherently interested in life; we all hunted for frogs in a pond as a kid. I thought biologists would have more trouble, but it was the other way around. Biologists have some quantitative training, and with some work, they can learn [the computational side]. The mathematicians and physicists don't like complexity. They like an algorithm."

Nathan Price is learning how to tread that path between disciplines. A 2005 graduate of the University of California, San Diego, bioengineering department, he accepted a faculty position at the University of Illinois but decided first to do a postdoc at ISB to gain a better understanding of systems biology. In graduate school, he primarily modeled metabolic systems; at ISB, he uses systems biology to analyze secreted bloodstream proteins that might act as early-stage fingerprints for cancer diagnosis. The work is computationally intensive, but his research drove him toward the bench. "You need to be able to go where the problem takes you," he says. You need to be able to do some basic experiments, he says, because it can be difficult to find people to do work that they might not find intellectually stimulating. Despite the premium on teamwork, "you handicap yourself if you always have to find a collaborator when you want to validate something."

Costs and benefits

No one doubts that the focus on working together is a good thing for biology, but is it good for a researcher's career? As the number of authors on a paper grows, it becomes more difficult for potential employers to distinguish an individual's role. "A paper with 30 authors can stand in the way of recruitment," says Brent.

Academic environments can be particularly hard on work that resulted from a team approach. Tenure committees, for example, tend to evaluate a faculty member on the ability to conduct solo research—the traditional mark of the competent scientist. "They have to bend a bit and make it possible for teams of young people to work together across departments and forge relationships—to be respected for that work even if they're members of a coalition. That's a work in progress. It's why MSI is not affiliated with a university," Brent says.

Getting the proper training is another challenge. Even 6 years after the founding of ISB, few academic departments specialize in systems biology. Training should start as an undergraduate, says Hood, who advises every biologist to get a second major in computer science or mathematics. Barnett urges graduate students to find an adviser who will let them expand beyond the tight focus of the typical Ph.D. project. "It takes a unique adviser to let them do that," he says.

It also takes a unique scientist. "It takes the right kind of people. Some people don't want to be this diverse," says Hood. Brent agrees that work in systems biology can be difficult, noting that potential hires at MSI are subjected to an intense process of evaluation: "A candidate has to be quite committed to put up with the stress of the coming years. We are not unpassionate about what we do."

–JIM KLING

Jim Kling writes for ScienceCareers.org from Bellingham, Washington.

EDITORIAL FEATURE

A Meeting of Minds, Expertise, And Imagination

European systems biology is pushing the boundaries between disciplines and cultures

CAMBRIDGE, U.K.—British systems biologist Eric de Silva—an astrophysicist by training—began his systems biology education "by sitting at home reading popular science books." Later, he says he "was brave enough to pick up [the textbook] *The Cell*," and his biology education began in earnest. De Silva now investigates protein interaction networks as a postdoc at Imperial College London.

De Silva's experience is typical. Few of today's systems biology postgrads, postdocs, and group leaders were trained as interdisciplinary scientists. Most acquired the skills they need to work and communicate with scientists from different disciplinary backgrounds on their own, informally. As they struggle to piece together pathways and networks and map out relationships among the components of biological systems, they must also piece together professional networks and discover new ways to work together. But for those who manage to bridge different fields, prospects are promising.

A model group. Edda Klipp's lab of modelers in Berlin sticks closely to experimentalists.

"It's a growth area and a young field with not a lot of senior people," says Rüdi Aebersold, a professor of systems biology at the Swiss Federal Institute of Technology Zurich (ETH Zurich) and the University of Zurich. "There's a great opportunity for young people starting out."

Although the United States is the pioneer and still the world leader in the emerging field, "systems biology in Europe is very dynamic," says Aebersold, one of the founding members of

CREDITS (TOP TO BOTTOM): DAVID PINCUS; K. GUENTHER

the Institute for Systems Biology in Seattle, Washington (see p. 1305). Aebersold returned to Europe at the end of 2004 and got involved with SystemsX, a collaboration between the universities of Basel and Zurich and ETH Zurich. Systems biology is a priority area in the European Commission's 7th Framework Programme, and the E.U. has just funded a €9 million pan-European systems biology project called Experimental Network for Functional Integration (ENFIN), among other projects. Most of Europe's national governments are making sizable bets—millions of euros—on systems biology projects. Large-scale collaborations are up and running, and more—such as SystemsX and ENFIN—are starting up. "In the way that molecular biology dominated the last half-century, systems biology will dominate the next half-century," predicts French systems biologist Nicolas Le Novère, a group leader at the European Bioinformatics Institute (EBI) in Hinxton, U.K. "Systems biology is here to stay."

De Silva. Moonlights as a biology student.

Building human networks

The systems biology workforce comprises classical experimental biologists, clinical scientists, mathematicians, computer scientists, physicists, engineers, and other specialists. Most research groups collaborate with researchers outside their specialties and often outside their own institutes. Edda Klipp heads the kinetic-modeling group at the Max Planck Institute for Molecular Genetics in Berlin; she is also an ENFIN partner. She says her group is "interested in trying to represent [biological] networks in mathematical terms" and in figuring out why certain features of a system evolve the way they do. Klipp's group is entirely theoretical, but she believes that "if you want to be close to nature, you need to have real data," so her group collaborates with several experimental groups. Some of her students have spent weeks or months in wet labs, learning new skills and cementing relationships.

Learning to collaborate—to get along with other scientists in a productive way—takes time, says Ewan Birney, a bioinformaticist at EBI and the coordinator of ENFIN. A big part of his role at ENFIN, he says, is "managing expectations: Experimentalists and theorists have different perspectives." It can be a difficult challenge, but it's essential. "Experimentalists have certain views," notes Klipp. "Models need different parameters; sometimes we"—theorists and modelers—"are aware of aspects they haven't discovered." Reality checks are also common the other way around. "We run our ideas by biologists," says de Silva, "and sometimes they rightly say, 'That is nonsense.' "

Sometimes the boundaries of communication are pushed. "Communication has always been one of the problems" of systems biology, says Le Novère. "It's not a dialogue between two disciplines; it's more like three or four." The key, say experienced systems biologists, is to be patient and give professional networks time. "At the start, you are kind of learning a language," says Klipp. Jörg Stelling, a systems biology group leader and assistant professor of bioinformatics at ETH Zurich, calls it "an investment." In his experience, learning to communicate adequately with collaborators "can take one to one-and-a-half years. But it's worth the effort," he adds.

Training as a systems biologist

Like de Silva, most of today's systems biologists come from traditional backgrounds and had to learn the systems biology ropes ad hoc. Le Novère trained in molecular and cellular pharmacology and taught himself computer programming and bioinformatics. His training, he says, "was not adequate." De Silva, who made the switch from astrophysics to biology, first got exposure to biology on the statistics end of a genetic-population project, which prompted him to read those biology books by moonlight.

Le Novère. Sees career potential for postdocs in systems biology.

So what kind of training, whether systematic or ad hoc, should aspiring systems biologists pursue? Stelling recommends "learning the basics in certain areas: stats, calculus, and linear algebra." Biologists "need to know how models can be set up." De Silva adds: "Be able to program." With these combined skills, he feels that researchers will be able "to analyze data sets themselves, quickly and easily." At the same time, "mathematicians and computer scientists need to acquire a biological way of thinking," says Stelling. "You need to learn the fundamentals in cell biology, molecular biology, and biochemistry."

Michael Stump, a group leader at Imperial College London and part of Imperial's new Centre for Integrative Systems Biology, advises students to "do what undergraduate degree you are most interested in, and do a master's course afterward." A number of master's-level courses that build bridges between disciplines have come on stream in recent years.

Gianni Cesareni—who has a hybrid computational and experimental lab at the University of Rome Tor Vergata and is part of the ENFIN initiative—believes that balance is key: "At the level of Ph.D., you need some specialist expertise, but you also need some interdisciplinary exposure." He recommends that researchers at the postgraduate level talk to students with different backgrounds. "Students need to go to common meetings," he says.

Job-market growth

Such training investments are likely to pay off for researchers with a talent for biological systems, because job opportunities are increasing rapidly. "At the moment, the mood is very good; there are a lot of things to do," says Klipp. And with funding levels high, the trend is likely to continue, say researchers in the field. Le Novère says that "the most striking effect is the number of new group-leader positions. It's one of the areas as a postdoc that you have a chance to become a group leader early."

But biologists who avoid mathematics should also avoid systems biology. "You need to have some maths mentality, and there are some students who deliberately pick biology because they want to avoid maths," says Cesareni. "It's not for everyone," says Stump—but he also predicts an era in biological research when "it will be hard to get a job if you are innumerate."

For Stump and ENFIN's Birney, these challenges are offset by substantial rewards. "It's a learning process, but great fun and adventure," says Stump. Birney adds, "It's a pleasure to coordinate; people want to collaborate."

As for de Silva, his appetite for biology is not yet quenched. "I feel I would be missing out a lot if I hadn't entered the world of biology. I thought there couldn't be anything as complicated as the universe until I started reading about the cell."

–ANNE FORDE

Anne Forde is Editor, North and East Europe, for ScienceCareers.org.

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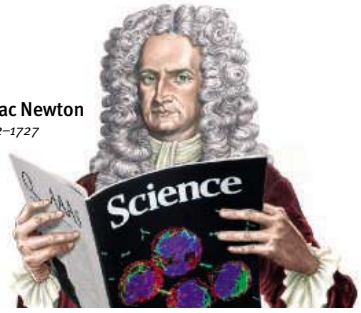

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If you are interested you must in the first instance contact a current member of the Sanger Institute Faculty **(http://www.sanger.ac.uk/Teams/faculty/)** to discuss potential projects.

The closing date for applications is 14 April 2006. Applications received after this date, or those which do not conform to the above criteria will not be accepted.

Questions regarding the process can be emailed to **humanresources@sanger.ac.uk** or by contacting Human Resources on telephone number: 01223 494882

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NATIONAL SCIENCE FOUNDATION
DIVISION OF INTEGRATIVE ORGANISMAL BIOLOGY

Computational Biologist (Program Director)
AD-401-4
Annual salary ranges from $91,407 to $142,449

The National Science Foundation's Directorate for Biological Sciences (BIO), Division of Integrative Organismal Biology (IOB) is seeking qualified candidates for one position as Program Director in computational biology. The Division (IOB) supports integrative research in emerging areas of organismal biology of plants and animals including neuroscience, developmental biology, ecological and evolutionary physiology, and behavior. Individuals with extensive cyber experience are especially encouraged to apply. More information about IOB can be found on their website at: **http://www.nsf.gov/div/index.jsp?div=IOB**.

Appointment to this position is to be filled as a permanent Program Director. Applicants must possess a Ph.D. in biology or in an equivalent discipline. In addition, six or more years of successful research, research administration, or managerial experience beyond the Ph.D. is required.

The announcement **E20060055**-Permanent, which includes position requirements and application procedures is located on NSF's Division of Human Resource Management website at **http://www.nsf.gov/about/career_opps/** or can be obtained by contacting **Myra Loyd** at **703-292-4363**. Hearing impaired individuals may call **TDD (703) 292-8044**. Applications must be received by **April 3, 2006**.

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Center for Biopreparedness and Infectious Disease
Department of Microbiology and Molecular Genetics
Tenure Track Position:
Microbial Pathogenesis and Host Response

MEDICAL COLLEGE OF WISCONSIN

Candidates with advanced degrees (MD, PhD, MD/PhD) are invited to apply for a tenure-track Assistant Professorship; outstanding individuals of higher rank will be considered. Investigators interested in the interaction of microbial pathogens with their mammalian hosts are encouraged to respond. We are particularly interested in scientists whose research centers on the mechanisms by which bacterial and viral pathogens interface with cellular functions and manipulate the innate or acquired immune response.

The Center for Biopreparedness and Infectious Disease (CBID) is part of campus wide initiatives to build research programs with relevance for the development of novel therapeutics and vaccines. Members of CBID and the Department of Microbiology and Molecular Genetics actively participate in the Great Lakes Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research. The successful applicant will join a highly interactive and collegial group of well-funded investigators and be expected to establish an independent research program that includes participation in graduate and medical student teaching. Competitive packages with salary support and start-up funds, newly constructed laboratory space and state-of-the-art BSL3 and ABSL3 core facilities will be provided.

Applications will be considered as they arrive but must be received by **May 1, 2006**. Applicants should submit a curriculum vitae, statement of research interests and the names of three references **c/o Cathi Kienast** to: **Dr. Dara W. Frank, Director, Center for Biopreparedness and Infectious Disease, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226, E-mail ckienast@mcw.edu; http://www.mcw.edu/microbiology**.

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NATIONAL CANCER INSTITUTE

POSTDOCTORAL POSITIONS AVAILABLE IN THE CANCER AND DEVELOPMENTAL BIOLOGY LABORATORY

Postdoctoral Positions In Nuclear Organization and Epigenetic Regulation. Postdoctoral positions are available to study the role of nuclear architecture and transacting factors regulating stem cell commitment. The projects will center on the introduction of mutations into nuclear envelope (NE) proteins, using gene manipulation techniques in mice and human ES cells, as well as the identification of trans-acting factors regulating stem cell differentiation. Opportunities will be available to utilize a wide range of molecular, cellular and genetic experimental approaches including microarrays, proteomics, imaging and gene targeting. A strong background in molecular biology and/or cell biology is required. *The Laboratory is located at the National Cancer Institute (NCI)-Frederick, Frederick, MD and is part of the National Institutes of Health (NIH), NCI, Center for Cancer Research (CCR). Interested applicants should send or email* ***(stewartc@ncifcrf.gov)*** *a cover letter, your curriculum vitae and three letters of recommendation to:* ***Dr. Colin Stewart, MDB/CDBL/CCR, NCI-Frederick, P.O. Box B, Frederick, MD 21702****.*

The NCI offers competitive Postdoctoral stipends and an excellent work environment.

Starting salary for these positions is $41,700 - $51,200.

NIH SCIENTIFIC REVIEW ADMINISTRATOR
(Health Scientist Administrator)
Vacancy: CSR-06-111051

Center for Scientific Review

We are seeking a qualified scientist, with doctorate level training and independent research experience in the neural basis of behavior to join a team of Scientific Review Administrators (SRAs) to help shape the future of scientific review. The incumbent will be responsible for the initial administrative and scientific review of NIH neuroscience research grant applications and will possess an M.D. or Ph.D. degree (or have equivalent training and experience), have independent research experience and a strong publication record. A broad knowledge of neuroscience is desirable, with a specific emphasis on the neural basis of behavior such as motivation and emotion. The position is in the Integrative, Functional, and Cognitive Neuroscience (IFCN) Integrated Review Group (IRG). This IRG is responsible for administering the review of a wide range of neuroscience research aimed at furthering our understanding of how the nervous system is organized and functions at an integrative, systems level. For additional information on the IRG please see our web site, at: http://cms.csr.nih.gov/PeerReviewMeetings/CSRIRGDescription/IFCNIRG/

Salary is commensurate with research experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life and long-term care insurance, Thrift Savings Plan participation, etc.) is available.

For additional information on this position, and for instructions on submitting your application, please see our website, at: http://jobsearch.usajobs.opm.gov/ listed under vacancy announcement number **CSR-06-111051**.

The closing date for this position is **March 17, 2006.**

WWW.NIH.GOV

National Institutes of Health
Office of the Director

Chief NIH Ethics Officer

The Office of the Director, National Institutes of Health (NIH) in Bethesda, Maryland, is seeking a Chief NIH Ethics Officer (CNEO), who will also serve as Director for the NIH Ethics Office. If you are an exceptional candidate with a M.D. and/or Ph.D. and have the vision and skills to oversee and provide strategic direction to NIH activities relating to ethics policy, oversight, and operations for scientific administration, we encourage your application.

To achieve its mission of advancing biomedical and behavioral research to improve the health of the public, the NIH must have the trust of the public that the decisions and activities of the agency and its employees are unbiased. The creation of the new position of CNEO is part of a comprehensive effort to strengthen the program of ethics oversight for NIH employees. As CNEO, you will be responsible for the executive leadership, strategic direction, and oversight of the scientific, clinical, and administrative activities of NIH staff as they relate to ethics policies. This includes: assuring compliance with Federal, departmental, and agency ethics laws, regulations, and policies that apply to the official-duty activities, outside activities, and financial holdings of NIH's 18,000+ employees; overseeing a rigorous program of quality control and risk management including assessing the effectiveness activities of conflict of interest operations the NIH Ethics Office and the application of delegated authority; conducting a comprehensive ethics training program; and developing/maintaining an effective enterprise information technology system. Additional functions include serving as the NIH spokesperson and principal advisor to the Director and Deputy Director, NIH on relevant NIH ethics policy and programs. In addition, you will serve as the NIH Agency Research Integrity Liaison Officer (ARILO) and will be responsible for all matters related to NIH's intramural and extramural research integrity programs to include oversight and coordination of NIH activities related to research misconduct and the promotion of research integrity of NIH intramural and extramural research programs. Understanding the value of scientific expertise for leadership of ethics at NIH, you will have the flexibility to devote up to 25% of your time to conduct or oversee research in an NIH intramural research laboratory or in an appropriate NIH extramural scientific programmatic role.

Salary is commensurate with experience; a full package of benefits is available, including retirement, health, life, long term care insurance, Thrift Savings Plan participation, etc.

Applications for this position will be reviewed by a Search Committee chaired by Dr. Duane Alexander, Director, National Institute of Child Health and Human Development. Applicants may send a Curriculum Vitae to **Teresa Leary, 31 Center Drive - MSC 2207, Room 4B-41, Bethesda, MD 20892-2207 or visit http://www.jobs.nih.gov and apply to Announcement OD-06-109779 (for Ph.Ds) or OD-06-109626 (for M.Ds)**. If you need additional information, please contact **Ms. Teresa Leary at learyte@od.nih.gov or (301) 496-1443**. Applications must be received by close of business **April 11, 2006**.

Job Information

Job Title: 7 Positions for a Health Science Administrator, GS-601-12/13
Employer: NIH/NIAAA (National Institute on Alcohol Abuse and Alcoholism)
Location: Rockville, MD
Date: 2/7/2006

NIAAA
National Institute on Alcohol Abuse and Alcoholism

Job Description

Description: HEALTH SCIENCE ADMINISTRATOR, GS-601-12/13

Department of Health and Human Services
National Institutes of Health
National Institute on Alcohol Abuse and Alcoholism

The National Institute on Alcohol Abuse and Alcoholism (NIAAA), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is recruiting 7 positions located in 4 Divisions —the Division of Metabolism and Health Effects (2 positions), the Division of Treatment and Recovery Research (1 position), the Division of Neuroscience and Behavior (2 positions), and the Division of Epidemiology and Prevention Research (2 positions).

The purpose of these positions are for the scientists to serve as a staff specialist for a significant segment of a subject-matter or program area (e.g. endocrinology, systems biology, animal and human behavioral neuroscience, social neuroscience, metabolism, behavioral treatment for alcohol use disorders, as well as alcohol epidemiology and prevention science) in which competent and definitive research is being conducted, and the research proposals have direct application to the NIAAA mission. Scientists at this level provide technical guidance to the scientific community in planning, coordinating, and evaluating proposed research projects and programs of interest to the NIAAA and conduct assignments in the major functional areas of administering research grants and contracts. The scientists judge the relative value of research being proposed or continued against specific goals. The scientist's knowledge about the field is that of one who is recognized as a competent researcher. These positions have overall responsibility for the goals, organization, administration, integrity, and conduct of the program. Perform of duties is concentrated in three major areas: program leadership and development, project management and administration, and scientific activities.

Applicants must have successfully completed all requirements for an M.D., Ph.D., or equivalent degree in a relevant biomedical or health-related field (e.g., behavioral neuroscience, biochemistry, molecular biology, physiology, psychology etc.) at an accredited university and have a strong record of research accomplishment demonstrating ability to collaborate with scientists in other disciplines, to conduct research, and a willingness to work in a team setting. Compensation will be commensurate with relevant work experience. Interested candidates should apply online through **USAJOBS.COM**, vacancy number **NIAAA-05-106373-DE**.

Department of Health and Human Services
National Institutes of Health
Office of the Director

Director, Office of Portfolio Analysis and Strategic Initiatives

The Office of the Director, National Institutes of Health (NIH) in Bethesda, Maryland, is seeking a Director for the new Office of Portfolio Analysis and Strategic Initiatives (OPASI). If you are an exceptional candidate with an M.D. and/or Ph.D. and the vision and ability to integrate science across multiple disciplines, we encourage your application.

As the OPASI Director, you will be responsible for the executive leadership for the coordination of overall NIH research portfolio analysis and strategic initiatives that fall within the OPASI's scope. The OPASI's primary objective is to develop: a transparent process of planning and priority-setting characterized by a defined scope of review with broad input from the scientific community and the public; valid and reliable information resources and tools, including uniform disease coding and accurate, current and comprehensive information on burden of disease; an institutionalized process of regularly scheduled evaluations based on current best practices; the ability to weigh scientific opportunity against public health urgency; a method of assessing outcomes to enhance accountability; and a system for identifying areas of scientific and health improvement opportunities and supporting regular trans-NIH scientific planning and initiatives. You will serve as the principal advisor to the NIH Director on issues involving OPASI's planning, analysis, and policy formulation and implementation activities, including efforts to strengthen trans-NIH strategic planning and funding, and improve data quality and develop analytical techniques for assessing the NIH research portfolio.

This position requires that the OPASI Director exercise leadership, initiative, and creativity in establishing and maintaining relationships with key Federal and non-Federal officials, nationally and internationally recognized scientific leaders and officials of academic, research, and other institutes and organizations, and professional and advocacy groups.

Salary is commensurate with experience; a full package of benefits (including retirement, health, life, long term care insurance, Thrift Savings Plan participation, etc.) is available.

Applications for this position will be reviewed by a Search Committee chaired by Dr. Jeremy Berg, National Institute of General Medical Sciences and Dr. Elizabeth Nabel, Director, National Heart, Lung and Blood Institute.

Interested applicants should send a Curriculum Vitae to **Ms. Teresa Leary, 31 Center Drive – MSC 2207, Room 4B-41, Bethesda, MD 20892-2207 OR visit: http://www.jobs.nih.gov and apply to Announcement OD-06-109905 (for Ph.Ds) or OD-06-109915 (for M.Ds).** If you need additional information, please contact **Ms. Teresa Leary at learyte@od.nih.gov or by calling 301-496-1443.**

Applications must be received by close of business April 11, 2006

Cancer Research Careers

Advertising Supplement

Be sure to read this special ad supplement devoted to cancer research opportunities in the upcoming **24 March issue of *Science*.**

You can also read it online on **www.sciencecareers.org.**

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Department of Health and Human Services
National Institutes of Health
National Institute on Aging

The National Institute on Aging, a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS) is recruiting for **four post-doctoral fellows** in the Laboratory of Genetics, Intramural Research Program (IRP):

1) with a background in cell based screens or imaging studies to work in the Image Informatics and Computational Biology Unit (IICBU), for high-throughput automated visual screening of RNAi libraries. The interdisciplinary group has developed image classification algorithms based on machine learning techniques, and we would like to apply these to the systematic reconstruction of genetic pathways. For additional information on this research, please go to: **(http://www.grc.nia.nih.gov/branches/lg/iicbu/iicbu.htm)**. Applicants should send the curriculum vitae, via email to Dr. Ilya Goldberg at, **goldbergil@grc.nia.nih.gov**.

2) with a background in biochemistry to work in the Transcription Regulation and Remodeling Section (TRRS), on purification of multi-protein complexes and analysis of their structures and functions **(http://www.grc.nia.nih.gov/branches/lg/trru/trru.htm)**. Projects include studies of chromatin-remodeling mechanisms (*G&D 19:1662-7*), DNA damage response, and human genomic instability diseases (*Nat. Genet. 35:165-170; 37: 958-63*). Applicants should send the curriculum vitae, via email to Dr. Weidong Wang, **wangw@grc.nia.nih.gov**

3) with a background in mouse development to work in the Developmental Genomics and Aging Section, to conduct the study of preimplantation mouse development (*Dev. Cell 6: 117-131, 2004*) and embryonic stem cells (*PLoS Biol. 1: 410-419, 2003*). The work utilizes embryogenomics approaches (*Trends Biotechnol. 19: 511-518, 2001*) and focuses on the identification and characterization of genes that are critical for the maintenance of pluripotency and/or for early commitment to different cell lineages. Applicants should send the curriculum vitae via email to Dr. Minoru Ko, **kom@grc.nia.nih.gov**.

4) with a background in molecular genetics to work in the Human Genetics Section, on the determination of skin appendage formation in vitro, based on signaling pathways operating with the EDA (ectodysplasin) TNF-ligand (*Hum. Molec. Genet. 11:1763-1773; Hum. Molec. Genet. 12: 2931-2940*). The aims include the understanding of how hair follicles form, as a model system for both development and possible regeneration. Approaches include histology, keratinocyte cell differentiation, and immunocytochemistry, as well as a range of genomic and physiological techniques. Applicants should send the curriculum vitae, via email to Dr. David Schlessinger, **schlessingerd@grc.nia.nih.gov.**

The successful individuals will possess an M.D. or Ph.D. degree in biochemistry, molecular genetics or a related field, with no more than five years of Post Doctoral research experience. Salary is commensurate with research experience and accomplishments.

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National Exposure Research Laboratory
Post-Doctoral Program

- The National Exposure Research Laboratory (NERL) of the United States Environmental Protection Agency is accepting applications beginning February 6 through April 7, 2006 for a number of federal, three-year post-doctoral research positions.
- Candidates will engage in research in areas such as environmental monitoring and characterization; computer modeling of the transport, transformation, and fate of pollutants in multiple media and at multiple scales; human and ecological exposure analysis; remote sensing applications; and landscape ecology.
- Specific research opportunities are posted on the NERL website at **http://www.epa.gov/nerl.**
- Post-doctoral positions will be in one or more of the following locations: Research Triangle Park, North Carolina; Cincinnati, Ohio; Las Vegas, Nevada; Athens, Georgia; or Washington DC metropolitan area.

FULL FEDERAL EMPLOYMENT BENEFITS:

- Salary range of $51,972 - $84,257
- Full three-year appointments
- Paid relocation to EPA duty location
- Federal health benefits, life insurance, and retirement program
- Flexible start date in 2006
- Vacation and sick leave
- Travel to professional and scientific meetings

APPLICATION PROCESS – Consult the NERL website at **http://www.epa.gov/nerl** for instructions on how to apply. Note – online applications are not accepted. Applicants must provide:

- Up-to-date Curriculum Vitae
- Letter of recommendation from your research advisor or comparable official
- Cover letter indicating: positions and locations of interest; your email address; U.S. Citizenship status, AND how you learned of this program
- DD-214, if claiming veteran's preference

Applicants must be United States citizens or permanent residents. Only in the absence of qualified U.S. citizens will permanent residents who are citizens of countries specified as exceptions to the appropriations act ban on paying non-U.S. citizens be considered.

Specific job information is posted on the NERL Internet site at **http://www.epa.gov/nerl**. EPA provides reasonable accommodations to applicants with disabilities. If you need a reasonable accommodation for any part of the application and hiring process, please notify the Agency. The decision on granting reasonable accommodation will be on a case-by-case basis.

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Science Careers Forum

- How long should it take to get my Ph.D.?
- Academia or industry?
- What will make my resume/cv stand out?
- How do I negotiate a raise?

Connect with Experts

Moderator Dave Jensen
Industry Recruiter

Mr. Jensen has over 20 years of experience in human resource consulting and staffing for the biotechnology and pharmaceuticals industry.

Adviser Bill Lindstaedt
Director,
UCSF Career Center

Mr. Lindstaedt has been providing career related advice to scientists and engineers for nearly 15 years, with a particular emphasis on working with graduate-level trainees in the life sciences.

Adviser Naledi Saul
Assistant Director,
UCSF Career Center

Ms. Saul has 7 years of career counseling with 4 years focused on counseling graduate students and postdocs in the biomedical and health sciences. Her forte is working with scientists pursuing careers in the public health arena.

Adviser Jim Austin
Editor, Science's
Next Wave

Dr. Austin has a Ph.D. in physics and worked in academia before coming on board to write about traditional and nontraditional career paths for scientists.

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Université de Neuchâtel unine

The University of Neuchâtel, Switzerland, seeks

A Full Professor of Analytical Chemistry specialised in Natural Products Chemistry

Description : Plant science is a research focus in the Biology Department. The successful candidate will develop a research program in natural products chemistry. He/she will collaborate with the National Center of Competence in Research "Plant Survival" and teach analytical and natural product chemistry.

Duties : Full chair (6 hours weekly teaching in French and English at the BSc, MSc and PhD levels, research, administration and analytical chemistry services).

Requirements : The successful candidate has an internationally recognized record of research in plant natural products chemistry including molecular biology.

Starting date : February 1, 2007 or to be convened.

Application : visit **http://www.unine.ch/sciences** under "emploi" for information.
The University of Neuchâtel encourages female applicants.

Applications deadline : April 30, 2006. The deadline may be extended. Send applications to the Dean: Prof. Dr. Jean-Pierre Derendinger, Chaire de chimie analytique, Secrétariat-décanat, rue Emile-Argand 11, CP 158, CH-2009 Neuchâtel. Tel :+41 32 718 2957
E-mail**: jean-pierre.derendinger@unine.ch.**
Please contact the Dean for details.

INSTITUTE OF LIFE SCIENCES - INDIA

Outstanding Research Positions

The **Institute of Life Sciences** (**ILS**) is a newly started not-for-profit venture at **Hyderabad, India** with the mission of finding solutions to improve human health through the application of cutting edge research in integrated disciplines of life sciences. Formed through the partnership of private industry, the State Government of Andhra Pradesh, and the University of Hyderabad, ILS will operate as an **autonomous body** and will pursue research in relevant areas of chemical and biological sciences in order to understand the molecular mechanisms behind chosen diseases and translate this understanding into approaches for discovering novel therapies. ILS is engaged in setting up a state-of-the-art center within the campus of University of Hyderabad. Research activities will commence in July 2006 in its equipped ready labs. More details about ILS are available at **www.ilsresearch.org**

ILS is now looking for bright **Ph D / M D** scientists at various levels of increasing accomplishments and responsibilities for recruitment as Senior Scientists, Principal Scientists and Principal Investigators. A **SENIOR SCIENTIST** will typically be a researcher with 3-5 years of postdoctoral experience and excellent record of publications/patents in areas of interest to ILS. A **PRINCIPAL SCIENTIST** is typically one who has 6-8 years experience and has independently formulated a viable research project, won grants for running it and found novel schemes, processes or products. A **PRINCIPAL INVESTIGATOR** is one who has over 10 years of proven expertise in conceptualizing projects, directing and managing research, and networking with thought leaders and other premier institutions in relevant fields of biomedical research. He/she would have a track record of independent research accomplishments in terms of grants, papers and patents, and motivating younger colleagues to aim and achieve.

For each of these positions, the compensation package will be generous and commensurate with experience and accomplishments. Interested candidates may send their resumes within the next 10 days by E-mail to Dr A Venkateswarlu, Director, ILS, at his email address: **venkata@ilsresearch.org**

THE UNIVERSITY OF CHICAGO

Lecturer/Lab Director Biophysics and Synthetic Biology

The Institute of Biophysical Dynamics invites applications for a non-tenure-track academic position as **LECTURER/LAB DIRECTOR** in a new cross-disciplinary graduate program in Biophysics and Synthetic Biology. The program, recently funded by the Howard Hughes Medical Institute, aims to transcend traditional boundaries and train a new generation of exceptional young scientists versed in both the physical and biological sciences. The Director is expected to help design, instruct and manage a novel, year-long laboratory course "From Synthesis to Measurement and Analysis of Biomolecular Function." A broad background and post-doctoral experience in biophysics is highly desirable.

Applications should include a CV, summary of previous experience, and teaching interests. Applicants must also arrange for three letters of reference. Review of applications will continue until the position is filled. Please submit applications to: **Tobin Sosnick, Search Committee Chair, Center for Integrative Science W225, 929 East 57th Street, Chicago, IL 60637.**

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.

STANFORD MATERIALS SCIENCE AND ENGINEERING

Faculty Position in X-Ray Studies of Materials Department of Materials Science and Engineering and Stanford Synchrotron Radiation Laboratory Stanford University

The Department of Materials Science and Engineering and the Stanford Synchrotron Radiation Laboratory Faculty at Stanford University invite applications for a junior level (Assistant or untenured Associate Professor) tenure-track position in the general area of x-ray investigations of materials and their properties. Applicants should hold an earned doctorate in a core engineering or science discipline and should have outstanding potential for establishing an independent research and teaching program. We are particularly interested in candidates who use advanced x-ray techniques associated with the study of nanoscale structures or ultrafast phenomena for the investigation of energy-related materials. Experience using x-ray probes for the study of materials and their dynamics is highly desirable. We expect the successful candidate to participate and contribute to the development and use of the outstanding x-ray facilities at Stanford, particularly the SPEAR-3 synchrotron storage ring, and the LCLS, which will soon be commissioned as the world's first x-ray free electron laser. In addition, he/she is expected to contribute to Stanford's multidisciplinary materials effort, and help to link this research on Stanford's main campus to research in the photon sciences at the Stanford Linear Accelerator Center.

We are especially interested in a person who could collaborate effectively with other materials science faculty and students engaged in research on energy-related materials and nano-technology, which are the core areas of the MSE department. We also seek an individual who is committed to excellence in teaching and to the mentoring of students. The successful candidate will be expected to contribute to the teaching program of the department by offering core courses in materials science, as well as by developing new curricula in, for example, materials characterization and x-ray based techniques and applications.

Applications should include a summary of their educational and professional background, a current list of published work, evidence of teaching experience and the names of at least three referees who may be consulted by the search committee. An indication of how the candidate's experience matches the position described above should also be given. Applicants are encouraged to write brief descriptions of their plans for future research and how those plans might be realized in a Stanford setting, as well as to submit a statement on teaching, focusing especially on their vision of teaching to students in the Department of Materials Science and Engineering. The appointment is expected to be made during 2006; applications (three copies) should be submitted by **May 15, 2006** to: **Professor Bruce M. Clemens, Co-Chair, MSE/SSRL Joint Search, Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305-2205; phone: (650) 725 - 7455; fax: (650) 725 - 4034; e-mail: clemens@soe.stanford.edu.**

Stanford University is an Equal Opportunity, Affirmative Action Employer.

ENFIN: *Enabling Systems Biology*

ENFIN is a Network of Excellence funded by the European Community, composed of 20 groups across 17 institutions in 13 European countries. The aim of ENFIN is to build a virtual institute that will place Europe at the center of the Systems Biology revolution. By combining the expertise of both "wet" and "dry" biologists, ENFIN will provide a platform incorporating computational approaches into the molecular biologist's tool set.

Currently available positions are:

- **Software developer**
  EMBL-EBI (Hinxton, United Kingdom)
- **Postdoctoral position in computational biology**
  Serono Pharmaceutical Research Institute, (Geneva, Switzerland)
- **Postdoctoral position in bioinformatics**
  Centre for Integrative Bioinformatics (Amsterdam,The Netherlands)
- **Postdoctoral position in bioinformatics**
  EMBL (Heidelberg, Germany)
- **Postdoctoral position in bioinformatics**
  Max-Planck Institute of Biochemistry (Martinsried, Germany)
- **Postdoctoral position in computational systems biology**
  University of Helsinki (Helsinki, Finland)
- **Postdoctoral position in computational biology**
  Genoscope (Evry, France)
- **Postdoctoral position in computational genomics**
  National Center for Cancer Research -CNIO (Madrid, Spain)
- **Postdoctoral position in bioinformatics**
  University of Dundee (Dundee, United Kingdom)
- **Bioinformatics researcher for systems biology**
  EGeen (Tartu, Estonia)

Positions details and contacts can be found at **WWW.ENFIN.ORG**

Faculty Position
Biological Oceanography

The Department of Oceanography at the University of Hawaii's Manoa campus is inviting applications for a full-time, 9-month, tenure-track position in biological oceanography, at the rank of assistant, associate or full professor. The successful candidate will be expected to develop outstanding research and teaching programs. The Department is part of the School of Ocean and Earth Science and Technology (SOEST) at the University of Hawaii at Manoa. We particularly seek applicants whose research complements our existing expertise, and the long-range plans for the school and the department. Among other priorities, these plans emphasize (1) integrative research on biological-geochemical or biological-physical interactions and the implications for climate, global change, or ecosystem health and sustainability and (2) developing the capacity for autonomous observations of organisms and biological processes in the sea. However, individual qualifications and excellence, rather than specific research discipline, will be the primary basis for the decision to hire.

MINIMUM QUALIFICATIONS: At the assistant professor rank, the Ph.D. degree in oceanography, marine biology, or related discipline; excellent communication skills; and demonstrated capability for creative, high-quality research. At the associate professor rank, the Ph.D. as above and five years of experience at the assistant professor level. At the full professor rank, the Ph.D. as above and four years of experience at the associate professor level. Apply in writing with supporting materials including (i) curriculum vitae, (ii) statement of research and teaching interests, (iii) three representative publications, and (iv) the names and addresses of three references. Send application to: **Biological Oceanography Search Committee, Department of Oceanography, University of Hawaii at Manoa, 1000 Pope Road, Honolulu, HI 96822**. Review of applications will begin **31 March 2006**. The position will remain open until filled. Questions may be addressed to **Dr. Grieg Steward, Department of Oceanography, 808-956-6775, grieg@hawaii.edu**.

The University of Hawaii is an Equal Opportunity/ Affirmative Action Employer.

UNIVERSITY OF MASSACHUSETTS AMHERST
ASSISTANT OR ASSOCIATE PROFESSOR IN "STEM CELL" AND "GERM CELL/DEVELOPMENTAL" BIOLOGY

The Department of Veterinary and Animal Sciences at the University of Massachusetts, Amherst (http://www.umass.edu/vasci) invites applications for two Tenure-Track faculty positions, at the Assistant /Associate Professor level. Applicants are required to have a Ph.D, or DVM/Ph.D, or MD/Ph.D, post-doctoral training and to have developed an independent creative research program in: (i) an aspect of "Stem Cell Biology" including but not limited to nuclear transplant cloning, organ culture and tissue regeneration, or (ii) "Germ Cell/Developmental Biology" including but not restricted to derivation, characterization and functional analyses of embryonic stem cells and reversal of genetic imprinting. The candidates will be expected to jointly develop upper level courses in "Stem Cell Biology", and "Stem Cell Culture". The Department of Veterinary and Animal Sciences places special emphases on faculty-student interaction, interdisciplinary activities and cooperation among faculty, and we share a fundamental commitment to teach and attract a diverse student body.

Review of applicants will begin on March 30, 2006 and will continue until the positions are filled. Applicants should send a letter of intent, statement of research interests, current curriculum vita and the names and contact information of three references to: **Dr. Samuel J. Black, Department of Veterinary and Animal Sciences, 314 Paige Lab, University of Massachusetts, Amherst, MA 01003.**

The University of Massachusetts is located in a college town in Western Massachusetts within 3 hours drive of New York and 2 hours drive of Boston. The region houses Amherst, Smith, Hampshire and Mount Holyoke Colleges and is recognized nationally as both a center of higher education and a scenic treasure offering extensive opportunities for summer and winter recreation.

The University provides an intellectual environment committed to providing academic excellence and diversity including mentoring programs for faculty. The University of Massachusetts is an AA/EO employer. Women and members of minority groups are encouraged to apply.

The **Department of Microbiology, Immunology and Biochemistry** at the **Northeastern Ohio Universities College of Medicine** invites applications for a **tenure-track faculty position** at the **Assistant Professor** level. We are seeking an individual investigating liver and/or biliary disease which could result from a variety of causes including inherited defects, metabolic disturbances, alcohol, toxins, diet or infectious agents. The successful candidate will be expected to establish an externally funded research program and collaborate with other investigators. The candidate will also contribute to the teaching and training of graduate students, medical students, and pharmacy students. Teaching responsibilities will be primarily in the area of biochemistry.

The new faculty member will join a department of active researchers housed in state-of-the-art facilities. The facility is designed to promote interactions among researchers and provide laboratory and office space for the successful candidate. The facility contains all the equipment necessary for molecular biology studies as well as tissue culture facilities, flow cytometer, electron microscope, confocal microscope, atomic force microscope, and much more. A generous start-up package will be provided. Additional information about the department can be found on the department web site: **http://www.neoucom.edu/audience/about/departments/MicroImmunoBiochem**.

Applicants must possess a doctoral degree and have had post-doctoral training in the appropriate area. Interested candidates should submit a curriculum vitae, a statement of research interests, future research goals and letters from three references to the address below. Review of applications will begin immediately and continue until the position is filled.

John Docherty, Ph.D.
Professor and Chair
Department of Microbiology, Immunology and Biochemistry
NEOUCOM
P.O. Box 95, State Route 44
Rootstown, OH 44272-0095

The college's dedication to excellence is complemented by its profound commitment to building and sustaining a culturally diverse academic community. Individuals from historically under-represented groups are encouraged to apply. NEOUCOM is an Equal Opportunity Employer and Educator.

Looking for a great science career?

Get the experts behind you.
Visit www.ScienceCareers.org

Your career is too important to leave to chance. So to find the right job or get career advice, turn to the experts. At ScienceCareers.org we know science. And we are committed to helping take your career forward. Our knowledge is firmly founded on the expertise of *Science*, the premier scientific journal, and the long experience of AAAS in advancing science around the world. Put yourself in the picture with the experts in science. Visit www.ScienceCareers.org.

POSITIONS OPEN

FACULTY POSITION IN CANCER BIOLOGY
University of Cincinnati
Department of Molecular Genetics
Biochemistry and Microbiology

The Department of Molecular Genetics, Biochemistry and Microbiology, in affiliation with the University of Cincinnati Cancer Center, has a faculty opening at the tenure-track **ASSISTANT/ASSOCIATE/FULL PROFESSOR** level. We seek candidates who are able to develop a highly competitive research program or who have already established such a program. Research should be in the area of Cancer Biology and may include DNA repair, genome stability, cell cycle regulation, chromosome and chromatin structure, oncogenes or tumor suppressor genes.

Our 25 full-time faculty maintain highly visible research programs in multiple areas including mutation, chromosome structure, differentiation, developmental and cancer biology, structural biology, signal transduction, membrane transport, viral and microbial pathogenesis and molecular and cellular immunology. Our graduate program includes 40 graduate students and an equal number of postdoctoral fellows train in the Department. Our structural biology program includes both nuclear magnetic resonance (NMR) and X-ray crystallography. We maintain core facilities for production of transgenic and knockout mice, electron, confocal and light microscopy, gene microarray analysis, proteomics, informatics, and imaging. These provide a breadth of research opportunities.

For further information, please see **website: http://www.molgen.uc.edu/logic/.** Applicants should submit curriculum vitae, a brief description of research, and the names of three qualified references to:

Jerry B. Lingrel, Ph.D.
Professor and Chair
Department of Molecular Genetics
Biochemistry and Microbiology
P.O. Box 670524
Cincinnati, OH 45267-0524

RESEARCH ASSOCIATE/ RESEARCH ASSISTANT
Wayne State University, Detroit, Michigan
Eugene Applebaum College
of Pharmacy and Health Sciences, Department of Fundamental and Applied Sciences

Research Associate/Research Assistant. The position is available immediately to study the molecular mechanisms of lipid-mediated oxidative stress. Experience in molecular biology, cell culture, and biochemical analysis is required. Salary is commensurate with the experience. Successful candidates are expected to have: a M.S. in molecular biology or biochemistry and/or B.S. in biology or chemistry. Interested candidates must apply online at **website: http://jobs.wayne.edu/>http://jobs.wayne.edu.** In addition, send curriculum vitae, names and addresses of three references to **e-mail: dseparovic@wayne.edu.**

Reference letters should be mailed to:

Dr. Duska Separovic
Department of Fundamental and Applied Sciences, Room 5142
Eugene Applebaum College of Pharmacy and Health Sciences
Wayne State University
259 Mack Avenue
Detroit, MI 48201

Date posted: February 15, 2006.

Wayne State University is an Equal Employment Opportunity/Affirmative Action Employer.

ZOOLOGY CURATOR. Illinois State Museum seeks Ph.D. Vertebrate Zoologist for collection and research programs including molecular approaches to systematics, population biology, biogeography, or natural history; database management; and educational programming. Salary based on experience. Benefits package included. For more information and application procedures see **website: http://www.museum.state.il.us/jobs/.** *Affirmative Action/Equal Employment Opportunity.*

POSITIONS OPEN

FACULTY POSITION
Quaternary Paleoecology
Climate Change Institute
University of Maine

We invite applications for a tenure-track faculty position in paleoecology/climate change at the rank of **ASSISTANT PROFESSOR** or **ASSOCIATE PROFESSOR.** We seek an individual with demonstrated ability to attract funding to support research on biological responses to climate change through time. The individual's research interests should complement the substantial interdisciplinary and international program in our Institute **(website: http://www.climatechange.umaine.edu).** The successful applicant will have a joint appointment in the Department of Biological Sciences, and will have a 70 percent research, 30 percent teaching appointment. We invite applicants working with Quaternary fossil groups (pollen, diatoms, chironomids, plant macrofossils) or with other forms of biogeochemical evidence that can be used to reconstruct or model Quaternary environments. A Ph.D. in an appropriate discipline is required, and two or more years of additional experience are strongly preferred. Applicants should send their curriculum vitae, a statement of current and future research interests, potential courses to be offered, and names and addresses of three references references to: **G.L. Jacobson, Chair, Search Committee, Climate Change Institute, 302 Bryand Global Sciences, University of Maine, Orono, ME 04469; e-mail: jacobson@maine.edu.** Review of applications will begin on March 27, 2006, with a desired starting date of September 1, 2006. *The University of Maine is an Affirmative Action/Equal Opportunity Employer.*

A **POSTDOCTORAL POSITION** in **BACTERIAL PATHOGENESIS** is available in the laboratory of **Dr. Arne Rietsch** at **Case Western Reserve University** to study virulence mechanisms in the bacterial pathogen Pseudomonas aeruginosa. We are focusing on aspects of regulation of type III secretion genes (**Rietsch et al.,** *Proc. Natl. Acad. Sci.* **102(22):** 8006-11, 2005), as well as structure/function aspects of the secretion machinery. Interested applicants must have a Ph.D. in microbiology, molecular biology, or a related field. Candidates must be well versed in standard recombinant DNA and molecular biological techniques. Experience with protein purification is a plus. We offer a competitive salary, as well as a stimulating and fun work environment. Applicants should send curriculum vitae, a letter of interest, and a list of three references by e-mail (as PDF) to **e-mail: pa-postdoc@case.edu** or by regular mail to:

Dr. Arne Rietsch
Department of Molecular Biology and Microbiology
SOM-W222A
10900 Euclid Avenue
Cleveland, OH 44106-4960

VISITING ASSISTANT PROFESSOR OF BIOLOGY. Wabash College invites applications for the position of visiting professor of biology for the 2006-2007 academic year. Ph.D. is preferred; all but dissertation considered. Area of primary expertise in biology is open. The successful candidate will participate in team-taught introductory biology courses for majors or non-majors and senior seminar (a capstone seminar course for biology majors). Send a letter of application, curriculum vitae, brief statements of teaching philosophy, graduate transcripts, and three letters of recommendation to:

Dr. David T. Krohne
Chair, Biology Department
Wabash College
P.O. Box 352
Crawfordsville, IN 47933

Review of applications will begin on March 17, 2006, and continue until the position is filled. No electronic applications accepted. Questions may be directed to **e-mail: krohned@wabash.edu.** Information about the College and Department is available at **website: http://wabash.edu.** *Wabash College, a liberal arts college for men, encourages applications from women and minorities. Equal Opportunity Employer.*

POSITIONS OPEN

FACULTY POSITION IN MICROBIOLOGY
University of Cincinnati
Department of Molecular Genetics
Biochemistry and Microbiology

The Department of Molecular Genetics, Biochemistry, and Microbiology has a faculty opening at the tenure-track **ASSISTANT/ASSOCIATE/FULL PROFESSOR** level. We seek candidates who are able to develop a highly competitive research program or who have already established such a program that will synergize with current research programs in the Department. Research should be in the area of microbial pathogenesis.

Our 25 full-time faculty maintain highly visible research programs in multiple areas including viral and microbial pathogenesis, molecular and cellular immunology, chromosome structure, differentiation, developmental and cancer biology, structural biology, signal transduction, membrane transport and cardiovascular biology. Our graduate program includes 40 graduate students and an equal number of postdoctoral fellows train in the Department. Our structural biology program includes both NMR and X-ray crystallography and the College has a state of the art BL3 containment facility for work with pathogens. We maintain core facilities for production of transgenic and knockout mice, electron, confocal and light microscopy, gene microarray analysis, proteomics biology, informatics, and imaging. These provide a breadth of research opportunities.

For further information, please see **website: http://www.molgen.uc.edu/logic/.** Applicants should submit curriculum vitae, a brief description of research, and the names of three qualified references to:

Jerry B. Lingrel, Ph.D.
Professor and Chair
Department of Molecular Genetics
Biochemistry, and Microbiology
P.O. Box 670524
Cincinnati, OH 45267-0524

ASSISTANT PROFESSOR. The Department of Biomedical Sciences (website: http://www.cvmbs.colostate.edu/bms) seeks to fill a tenure-track position at the rank of Assistant Professor. Candidates with expertise in cardiovascular/pulmonary physiology are especially encouraged to apply. The selected individual will be expected to establish an independent research program, teach systems-oriented physiology and related topics to professional veterinary students, and participate in teaching related topics to graduate and undergraduate students. Applicants must have an earned doctorate and postdoctoral research experience. A veterinary degree is not essential, but the successful candidate must be capable of teaching in a medically-oriented curriculum. A letter of application, curriculum vitae, statements of research and teaching interests, and list of three references who may be contacted when appropriate, should be sent electronically or by post to: **Dr. Richard Bowen, Animal Reproduction and Biotechnology Laboratory, Campus Delivery 1683, Colorado State University, Fort Collins, CO 80523-1683; e-mail: richard.bowen@colostate.edu.** Review of applications will begin April 15, 2006, and continue until a suitable candidate is found. *Colorado State University is an Equal Opportunity/Affirmative Action Employer.*

A **RESEARCH SCIENTIST** position is immediately available to study HIV and drugs of abuse. Candidate should have a Ph.D. with a strong background in cellular/molecular biology, immunology, genomics, and proteomics. Independent research studies in HIV and drugs of abuse are expected. Candidate must have a minimum of three years of molecular biology and molecular immunology research laboratory experience.

Please send curriculum vitae and two references to: **Dr. Madhavan Nair, Division of Allergy/Immunology/Rheumatology, Buffalo General Hospital, University at Buffalo, 100 High Street, Buffalo, NY 14203;** or **e-mail: cdsperry@buffalo.edu.**

POSITIONS OPEN

TEAM LEADER
Molecular Epidemiology and Bioinformatics Laboratory Branch, Division of Viral Hepatitis Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC), Division of Viral Hepatitis (DVH), Atlanta, Georgia, is seeking applications for a senior level Laboratory Scientist or Medical Officer with hepatitis experience to serve as Leader of its Molecular Epidemiology Team. The DVH serves as a World Health Organization Collaborating Center. The Team Leader provides leadership, direction and oversight to approximately three full-time equivalent positions, and 12 additional visiting scientists, guest researchers, and laboratory staff. Applicants must be qualified to oversee a comprehensive investigative agenda that includes transmission studies of all viruses causing hepatitis, A-E, as well as new and potentially pathogenic viruses. The Team works closely with other three teams in the Branch (Reference Diagnostics, Developmental Diagnostics, and Experimental Pathology) and also with the Epidemiology and Prevention Branches to accomplish the overall mission of the DVH. The Team Leader must possess a broad range of scientific knowledge and laboratory skills relating to the molecular biology, pathogenesis, virology, and immunology of hepatitis viruses, expertise in computation and quantitative analyses, and a comprehensive grasp of literature pertaining to hepatitis as well as scientific approaches taken by professional peers in government, industry, and academia. Candidates for the position must meet qualifications for the GS-14 level for the federal civil service and/or have a doctoral degree in medicine, microbiology, biology, or other appropriate field. Salary is commensurate with experience.

For more information contact **Chong-Gee Teo, M.D., Ph.D.** at **e-mail: enz0@cdc.gov,** or **Wendi Kuhnert, Ph.D.** at **e-mail: wdk1@cdc.gov. Telephone: 404-639-3103.** *CDC is an Equal Opportunity Employer.*

POSTDOCTORAL POSITIONS
Molecular Cell Biology of Diabetic Complications

As reviewed in *Nature* **414:** 813, 2001, our laboratory focuses on the mechanisms by which hyperglycemia causes vascular damage. We are currently investigating (a) the molecular basis for "metabolic imprinting;" (b) the genetic basis for familiar clustering of susceptibility to hyperglycemic damage; (c) endothelial progenitor cell dysfunction and impaired vasculogenesis in diabetes; and (d) identification of novel therapeutic strategies for preventing metabolite-induced vascular damage. Candidates should have a strong foundation in molecular and cell biology. (See **Yao, D. et al.,** *Cell* **124:** 275286, 2006.) Please send curriculum vitae and names and contact information of three references to: **Dr. M. Brownlee, Diabetes Research Center, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx, NY 10461; e-mail: brownlee@aecom.yu.edu.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITIONS

One to two postdoctoral positions are available at Duke Medical Center to use novel genomic tools and advanced bioinformatics to investigate tumor microenvironments (**Chi et al.,** *Pub. Lib. of Sci. Med.* **3:** e47, 2006). We look for ambitious and motivated individuals to join us at Institute for Genome Sciences and Policy (IGSP) in brand new Center for Interdisciplinary Engineering, Medicine, and Applied Sciences (CIEMAS) building. IGSP is a new institute established to tackle many issues with human diseases which are multidisciplinary in nature. The candidate is expected to have proven productivity and expertise with mammalian cell culture/molecular biology during the graduate training. Prior experience with microarray data and interest in bioinformatics will be a plus. Please send curriculum vitae and three references to **Dr. Jen-Tsan Ashley Chi** at **e-mail: chi00002@mc.duke.edu.**

POSITIONS OPEN

DISTINGUISHED FELLOW ON ENERGY POLICY

The University of Tennessee is seeking a distinguished and experienced professional in the field of energy to fill the new position of Fellow in the Howard H. Baker Jr. Center for Public Policy. The Fellow will be expected to lead research on our nation's energy policy, to organize and participate in activities that stimulate debate on critical policy issues related to energy, to be involved in teaching and related academic activities, and to publish the results of work growing out of these activities. An advisory board that includes individuals from the University of Tennessee, the Tennessee Valley Authority, and the Oak Ridge National Laboratory will assist the Fellow as appropriate. The goal of this activity is to make progress in the efforts to provide our nation with sufficient energy to meet its needs in an economically and environmentally acceptable manner.

Applicants should have appropriate advanced degrees, an international reputation in their field, and documented accomplishments in the field of energy. In particular, the Fellow should have extensive knowledge of energy production, consumption, and delivery, as well as the research and development needed to advance the state-of-the-art in the various fields of science, technology, policy, environment, and economics that intersect energy supply and demand issues.

The Howard Baker Center for Public Policy began operations in 2003 at the University of Tennessee in Knoxville. The Baker Center develops programs and promotes research to further the public's knowledge of our system of governance and of critical public policy issues, and to highlight the importance of public service.

The salary range is $150,000 to $200,000, determined by the qualifications and experience of the candidate. Benefits, housing, and travel are also included. It is anticipated that the Fellow will be appointed to a one-year (or more) term. The Fellow will be based at the Baker Center on the campus of the University of Tennessee in Knoxville, and would be expected to interact with the Tennessee Valley Authority and the Oak Ridge National Laboratory.

Interested applicants are encouraged to submit a letter of application, resume, and sample articles to:

Dr. Alvin Trivelpiece
Chair, Distinguished Fellow Search Committee
Howard Baker Center for Public Policy
The University of Tennessee
Room 217, Hoskins Library
Knoxville, TN 37996

The University welcomes and honors people of all races, genders, creeds, cultures, and sexual orientations, and values intellectual curiosity, pursuit of knowledge, and academic freedom and integrity.

The University of Tennessee is an Equal Employment Opportunity/Affirmative Action/Title VI/Title IX/Section 504/ADA/ADEA institution in the provision of its education and employment programs and services.

RESEARCH SCIENTIST

The Department of Medicine and James P. Mara Center for Lung Disease at Street Luke's-Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, invites applications for a Research Scientist position. We seek candidates with a Ph.D. or M.S. degree in organic chemistry or biochemistry. Applicants working in protein chemistry and/or liquid chromatography/mass spectrometry (LC/MS) are particularly encouraged to apply. Submit letter of application and curriculum vitae to:

Gerard M. Turino, M.D.
Department of Medicine/James P. Mara Center for Lung Disease
St. Luke's-Roosevelt Hospital Center and Columbia University College of Physicians and Surgeons
1000 Tenth Avenue, New York, NY 10019
E-mail: gmt1@columbia.edu
Telephone: 212-523-5919; fax: 212-523-3416

St. Luke's-Roosevelt Hospital Center is an Equal Opportunity Employer and welcomes applications from women and minority group candidates.

POSITIONS OPEN

PRINCIPAL INVESTIGATOR
Systems Biology

Genstruct is seeking Ph.D. (or M.D.) level Principal Investigators (PI) to lead multi-disciplinary teams in systems biology. As PI, you will be the primary scientific interface between Genstruct and our partners, and be responsible for the scientific outcome of research collaborations. You will work with partner Scientists to define research goals, then develop and execute project plans based on available resources.

PIs will become proficient in Genstruct's proprietary methodology and technology for causal systems modeling, pathway analysis, mechanism definition, and hypothesis generation and refinement. You will build, manage, and mentor your team to ensure quality and efficiency. Additionally, you will develop and present results at partner meetings and scientific conferences.

The ideal candidate will have excellent leadership, verbal communication and writing skills, as well as experience with high throughput biology methods, experimental designs, and computational analysis tools used to support the development and testing of mechanistic hypotheses. Research experience in cancer, metabolic disorders, and/or inflammation is preferred.

In lieu of a cover letter, please draft a single paragraph describing how you developed testable hypotheses based on experimental data. Include how the outcome(s) influenced your organization. This, along with curriculum vitae, should be e-mailed to **e-mail: career@genstruct.com.**

For the latest job postings, visit us on the web at **website: http://www.genstruct.com/careers.**

The Department of Biology at Indiana University Northwest (IUN) invites applications for a tenure-track position in **PLANT CONSERVATION BIOLOGY/GENETICS** at the level of **ASSISTANT PROFESSOR.** Candidates must have a Ph.D.; postdoctoral experience is a plus. The individual will contribute to our B. S. in biology and to redeveloping the general education curriculum, and may contribute to developing a proposed M. S. in biotechnology (e.g. plant conservation genetics/plant molecular biology) and a proposed M. S. in environmental studies. Applicants using molecular biological approaches to study of plant conservation biology earn a plus. Excellence in teaching at different levels is required. Northwest Indiana features the floristically diverse Indiana Dunes region lying at the junction of prairie, savanna/woodland, eastern deciduous forest, and northwoods ecoregions with diverse wetland types throughout. A 55-acre nature preserve is being restored adjacent to campus; on campus we have a tropical greenhouse.

Applicants should send curriculum vitae, statement of research interests, statement of teaching philosophy and interests, and arrange for three letters of recommendation to be sent to: **Dr. Spencer Cortwright, Chair, Department of Biology, Indiana University Northwest, Gary, IN 46408.** Review of applicants will begin March 15, 2006, and continue until the position is filled, with an expected start date in August 2006. *Affirmative Action/Equal Employment Opportunity Employer.*

POSTDOCTORAL POSITION in NIH-funded laboratory to study gastrointestinal mucosal immunology and carcinogenesis. Expertise in molecular biology required, microbiology or immunology background desirable. E-mail curriculum vitae and names of references to: **Keith T. Wilson, M.D., Professor of Medicine and Cancer Biology, Director of Research, Division of Gastroenterology, Vanderbilt University School of Medicine, Nashville, TN; e-mail: keith.wilson@vanderbilt.edu.** *Equal Opportunity Employer.*

cancer conference

ncri

national cancer research institute

NCRI
National
Cancer
Research
Institute

Second NCRI Cancer Conference
International Convention Centre, Birmingham, UK
8 – 11 October 2006

Abstract submission for the Second NCRI Cancer Conference opens on Monday 6 March 2006.
The deadline for abstract submissions is Sunday 7 May 2006.

Registration opens: Thursday 1 June 2006.

The National Cancer Research Institute (NCRI) is pleased to announce the second in a series of meetings that aim to provide the major forum for the dissemination of research advances in cancer, across all disciplines. The series is the biggest of its kind to be held in the UK.

The establishment of the NCRI Cancer Conference has brought together for the first time the major partners of the NCRI and cancer researchers from across the full range of disciplines. The first Conference was a great success, providing a unique opportunity in the UK for networking, debate and the generation of new ideas and collaborations.

The second NCRI Cancer Conference will include a wide variety of sessions that should appeal to cancer researchers from a wide range of disciplines, at all stages of their careers. These will mix high quality plenary and parallel sessions with focused satellite meetings and a range of workshops including; careers sessions for PhD students, post docs, clinical fellows, radiographers and research nurses; women in science; grant-writing; and educational sessions. As well as talks from internationally renowned speakers, we will be hearing directly from patients affected by the disease and how they are contributing to the development of the research agenda. The meeting will also include an event designed to engage and enthuse the public about cancer research.

Confirmed Plenary Speakers

Leslie Bernstein
Chris Boshoff
Harry Burns
Titia de Lange
Elizabeth Eisenhauer
Tariq Enver
Lesley Fallowfield
Richard Gilbertson
Gerard Hastings
Ken Hillan
Tyler Jacks
Scott Lowe
Gillies McKenna
Tom Smith
Fiona Watt

Parallel Session Themes

Cancer Cell Biology • Clinical • Epidemiology and Prevention • NCRI Clinical Studies Groups • Patients and The Public • Supportive and Palliative Care • Therapeutic Development

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Programme Planning Committee:
Alan Ashworth (Chair), Rob Coleman, Jessica Corner, Roger Griffin, Donna Johnstone, Rick Kaplan, Stan Kaye, Tony Kouzarides, Nick Lemoine, Roger Wilson

Parallel Session Topics: **Cancer Cell Biology:** Cancer genetics, Chemical biology in relation to cancer, DNA repair, Epigenetics and methylation, High throughput SNP screening for the identification of cancer genes, Immunology, Infection and cancer, NCRI informatics, Radiobiology, Stem cells in cancer, Tissue banks, Tumour cell migration and invasion, **Clinical:** Advances in upper GI cancer, Breast cancer, Colorectal cancer, Haemato-oncology, Imaging, Future directions in oncology imaging, Lung cancer, Ovarian cancer, Paediatric oncology, Prostate cancer, **Epidemiology and Prevention:** Behaviour change, Chemoprevention, Diet and cancer, **NCRI Clinical Studies Groups:** To be announced, **Patients and The Public:** Demonstrating value: patients in cancer research, Survivorship, Research information for patients and the public, **Supportive and Palliative Care:** Psychological approaches to reducing cancer risk and increasing survival, Addressing patients' care needs through nurse-led research, Places of care near the end of life, Primary care, **Therapeutic Development:** Immunotherapy, Titles of two further sessions to be confirmed.

NCRI Conference Secretariat PO Box 49709 61 Lincoln's Inn Fields London WC2A 3WZ United Kingdom
t: +44 (0)20 7269 3420 e: ncriconference@ncri.org.uk www.ncri.org.uk/ncriconference/

POSITIONS OPEN

FACULTY POSITION(S)
Molecular and Cell Biology

The Georgia Institute of Technology, one of the consistently top ranked educational/research institutions in the country, is undergoing significant growth in the biological sciences **(website: http://www.biology.gatech.edu)**. In the College of Sciences, the School of Biology is recruiting faculty in molecular and cell biology, and is particularly interested in candidates with expertise in developmental cell biology as part of the School's expansion in integrative systems biology.

It is anticipated that these faculty positions will be filled at the junior level but interested senior level candidates are also strongly encouraged to apply.

Applications should include a cover letter with a summary of the candidate's interests and plans, full curriculum vitae and contact information for four references, and be sent to: **John F. McDonald, Chair, School of Biology, Georgia Institute of Technology, 310 Ferst Drive, Atlanta, GA 30332.**

Georgia Tech is a unit of the University System of Georgia and an Affirmative Action/Equal Opportunity Employer and requires compliance with Immigration Control Reform Act of 1986.

POSTDOCTORAL POSITIONS are currently available in the **Molecular Neuroscience and Vascular Biology Laboratory** at the **University of Kentucky Medical Center,** Lexington, Kentucky, United States, to study molecular and cellular mechanisms of cell injury in relationship to disruption of the blood-brain barrier, tumor metastasis, and/or spinal cord trauma. Strong background in molecular biology is required. Please submit curriculum vitae and the names and telephone numbers of three references to**: Dr. Michal Toborek, University of Kentucky, Department of Surgery/Neurosurgery, 593 Wethington Building, 900 S. Limestone, Lexington, KY 40536, U.S.A. Fax: 859-323-2705; e-mail: michal.toborek@uky.edu.**

POSITIONS OPEN

POSTDOCTORAL POSITION
Indiana University

Research focus will be on the functions and behaviors of intrinsically unstructured proteins that are being uncovered in structural genomics efforts. The preferred applicant will have expertise in molecular biology, protein purification, and spectroscopies (fluorescence, circular dichroism) as applied to analysis of intrinsically unstructured protein. Interest or abilities in computational biology/bioinformatics would be desirable. There will be an opportunity to collaborate with a small biotechnology company. Please send cover letter and curriculum vitae and have three reference letters sent to:

A. Keith Dunker
Department of Biochemistry and Molecular Biology
Center for Computational Biology and Bioinformatics
Indiana University School of Medicine
714 N. Senate Avenue, Suite 250
Indianapolis, IN 46202

Indiana University is an Equal Employment Opportunity/Affirmative Action Employer, Minorities/Women/Persons with Disabilities.

The American Museum of Natural History is seeking a **POSTDOCTORAL ALGORITHM SCIENTIST** with a Ph.D. in computational science to perform research and implementation of algorithms for full-genome phylogenetic and biogeographic analysis of the evolution of diverse genomic systems. Other requirements include: experience in algorithm design, especially combinatorial optimization problems and programming skills for prototyping. Experience in string and parallel algorithms and computational biology desired. Term for one year. Fax curriculum vitae to **fax: 212-769-5277.** *Equal Opportunity Employer.*

POSITIONS OPEN

POSTDOCTORAL FELLOW
Wayne State University, Detroit, Michigan
Eugene Applebaum College of Pharmacy and Health Sciences
Department of Fundamental and Applied Sciences

Postdoctoral Fellow. The position is immediately available to study the molecular mechanisms of lipid-mediated oxidative stress. Experience in molecular biology, cell culture, and biochemical analysis is essential. The successful candidate will be able to communicate research data clearly via oral presentations and publications. Salary is commensurate with the experience. Interested candidates with a Ph.D. in molecular biology or biochemistry should send curriculum vitae, names and addresses of three references to **e-mail: dseparovic@wayne.edu.** Reference letters should be mailed to:

Dr. Duska Separovic
Department of Fundamental and Applied Sciences, Room 5142
Eugene Applebaum College of Pharmacy and Health Sciences
Wayne State University
259 Mack Avenue
Detroit, MI 48201

Date posted: February 9, 2006.

Wayne State University is an Equal Employment Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITION, Miami University, Oxford, Ohio, to study neuron-founder cell interactions during adult myogenesis in Drosophila. Opportunities to teach courses in cell and developmental biology. Candidates must have a Ph.D. and background in molecular genetics. Review of applications will begin March 13, 2006. Send a letter of application, curriculum vitae, and three letters of reference to **Dr. Joyce Fernandes, e-mail: fernanjj@muohio.edu.** *Miami University is an Equal Opportunity/Affirmative Action Employer.*

MARKETPLACE

MARKETPLACE

MARKETPLACE

“THE FASTEST GROWING CROP IN IOWA IS NOT WHAT YOU EXPECT.”

MORE AND MORE BIOSCIENCE COMPANIES PLANNING TO EXPAND ARE TAKING A LOOK AT IOWA. And for good reason. Iowa leads the nation in the production of raw biomass at roughly 2.75 billion bushels. The state is home to three public universities that are world-renowned for their research in plant, animal and human bioscience. And to date there are more than 1,800 Iowa establishments already involved in the bioscience industry. To learn more about expanding your business in Iowa, visit iowalifechanging.com. Because the closer you look, the more Iowa grows on you.

www.iowalifechanging.com

IOWA

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